

PHYTOCHEMICAL, PHARMACOLOGICAL EVALUATION AND STANDARDIZATION OF SELECTED MEDICINAL PLANTS FOR ANTI-INFLAMMATORY AND ANTI ARTHRITIC ACTIVITY

THESIS

SUBMITTED IN FULFILMENT OF THE REQUIREMENTS OF THE

DEGREE OF

DOCTOR OF PHILOSOPHY

SUBMITTED BY

ROHIN CHAUHAN

(GUUK/01/Ph.D./11/16)

SUPERVISED BY

Dr. MANOJ GHALOT

Senior Professor & H.O.D



SIRI GURU RAM RAI COLLEGE OF

PHARMACY, H.N.B GARHWAL

UNIVERSITY

DEHRADUN

NOV - 2016

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H.N.B GARHWAL UNIVERSITY
DEHRADUN, UTTARAKHAND, INDIA
(NOV- 2017)**

DECLARATION BY THE STUDENT

I hereby certify that the work which is being presented in this thesis entitled **“Phytochemical, Pharmacological Evaluation and Standardization of Selected Medicinal Plants for Anti-inflammatory and Anti-arthritis activity”** is for fulfillment of the requirement for the award of Degree of **Doctor of Philosophy** submitted in the **Guru Ram Rai college of Pharmacy, H.N.B Garhwal University, Dehradun, Uttarakhand** an authentic record of my own work carried out under the supervision of **Dr. Manoj Ghalot**, Senior Professor & H.O.D , SGRR College of Pharmacy, Garhwal University , Uttarakhand.

The work has not formed the basis for the award of any other degree or diploma, in this or any other Institution or University. In keeping with the ethical practice in reporting scientific information, due acknowledgements have been made wherever the findings of others have been cited.

Date:

ROHIN CHAUHAN

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the thesis entitled “**Phytochemical, Pharmacological Evaluation and Standardization of Selected Medicinal Plants for Anti-inflammatory and Anti-arthritis activity**” submitted by **Rohin chauhan Regd. No. GUUK/01/Ph.D./11/17** to the H.N.B Garhwal University, Uttarakhand in fulfillment for the award of the degree of **Doctor of Philosophy** is a *bona fide* record of research work carried out by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institution or University for the award of any degree or diploma.

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Outcomes of Doctoral Research

1.0 List of Publications for Doctoral research

1. **Rakesh K Sindhu and S Arora.** Herbs for the treatment of Arthritis and inflammation: A review. *Planta Activa*, **2012**, 3, 152-157.
2. **Rakesh K Sindhu and S Arora.** Phytochemical and Pharmacognostical studies on *Murraya koenig L . spreng* roots. *Drug Invention Today*. **2012**, 4(1), 325-336. **(Impact Factor 0.64).**
3. **Rakesh K Sindhu and S Arora.** Evaluation of phenolic contents and antioxidant potential of *Murraya koenigii (L)* spreng roots. *Journal of Applied Pharmaceutical Science*, **2012**, 2(11), 220-222.
4. **Rakesh K Sindhu and S Arora.** Therapeutic effect of *Ficus lacor* aerial roots of various fractions on Adjuvant-Induced Arthritic rats. *ISRN Pharmacology*, **2013**, 1-8.
5. **Rakesh K Sindhu and S Arora.** Phytochemical and Pharmacognostical investigations on *Ficus lacor* aerial roots. *International Journal of Phytomedicine*, **2013**, 5(3), 267-277. **(Impact Factor 1.09).**
6. **Rakesh K Sindhu and S Arora.** Free radical scavenging and antioxidant activity of *Ficus lacor* Buch. Hum. *Asian Journal of pharmaceutical and clinical research*. **2013**, 6(5). 184-186. **(Impact Factor 0.70).**
7. **Rakesh K Sindhu and S Arora.** Anti-inflammatory potential of various extract fractions of *Ficus lacor* aerial roots and *Murraya koenigii* roots. *Archives of Biological Sciences*. **2014**, 66 (3), 1261-1270. **(Impact Factor 0.607).**

2.0 List of Conferences Presentations

Sr. No	Dates	Conferences (National/International)	Title	At
1.	22-24 May, 2012	International (Poster Presentation)	Standardization and Phytochemical evaluation of <i>Murraya koenigii</i> roots	Rayat Institute of Pharmacy, Ropar, Punjab
2.	21-23, Oct, 2013	International (Poster Presentation)	Phytochemical and standardization of <i>Ficus lacor</i> aerial roots	Chitkara College of Pharmacy, Rajpura. Punjab
3.	17-18 Oct. 2014	National (Oral Presentation)	Potent anti-inflammatory activity of different extracts isolated from aerial roots of <i>Ficus lacor</i> Buch.-Ham.	MM College of Pharmacy, MMU, Mullana, Haryana

ABSTRACT

Objectives: The present thesis entitled “Phytochemical, Pharmacological Evaluation and Standardization of Selected Medicinal Plants for Anti-inflammatory and Anti-arthritic activity”. Materials and Methods: The *Murraya koenigii* roots collected from Chitkara University and *Ficus lacor* aerial roots collected from Panchkula, Haryana plants and identified and authenticated by Dr. H. B. Singh, Head, raw material and herbarium, NISCAIR, Delhi. Results and Discussion: The phytochemical and pharmacognostical evaluation of *Murraya koenigii* roots were performed. The root is a typical root and in transverse section it shows the features of a dicot root i.e. Epidermis, Cortex, Endodermis, Phloem and Xylem. The microscopy of the powder revealed the presence of xylem vessel, fibre, parenchymatous cell and cork cells. Total ash, acid insoluble ash, water insoluble ash and sulphated ash were 11.25%, 10.57%, 8.75%, 7.025% respectively. The extractive values i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were 3.5 %, 5.3 %, 3.2%, 8.5%, 4.5%. The fibre content was 7.53%. The plant can be used as bitter as its bitterness was found to be 2.5 unit/g. The plant possesses haemolytic activity. The plant extracts were good to be free of microbial contamination. The tannin content was 19. The alcoholic and aqueous extracts were screened for presence of amino acid and carbohydrates. The extracts showed the presence of two amino acids viz. Phenylalanine and glycine and two carbohydrates i.e. galactose, ribose and fructose. The preliminary phytochemical screening of Pet. Ether extract, Ethyl acetate extract, Chloroform extract, Ethanol extract, Aqueous extract was performed. The presence of alkaloids, flavonoids, carbohydrates, and sterol in various extracts were observed. This is first ever pharmacognostical study carried out on the aerial roots of *Murraya koenigii*. Three compounds isolated (i.e. 9-carbethoxy-3-methyl carbazole, 3-methyl carbazole and Koenoline) using different solvent in column chromatography, but these were already reported compounds. MKPE, MKEA and MKCF extracts from *Murraya koenigii*, both MKPE, MKCF and MKAF-2 were found to be significant in reducing inflammation similar to standard drug in different animal models of inflammation. MKEA was however no significant results showed. MKPE, MKCF and MKAF-2 showed statistically significant inhibition of arthritic lesions ($p<0.05$) from day 16, ($p<0.01$) from day 20 and ($p<0.001$) from day 21 onwards. The extracts administered in higher doses reduced the lesions to a greater extent showing a dose dependent decrease in lesions. The extracts MKPE and MKCF showed significant

increase in body weight ($p<0.001$) as compared to arthritic control group and increase in liver weight ($p<0.01$), decrease in liver weight ($p<0.001$) and increase in spleen weight ($p<0.001$) in arthritis control. MKEA not showed any significant result in body and organ weight estimation. The extracts MKPE and MKCF showed significant decrease in WBC count ($p<0.001$), increase in hemoglobin contents and RBC counts as compared to control group. The extracts MKPE and MKCF showed significantly ($p<0.001$) decreased the level of acid phosphatase in blood, whereas they also significantly ($p<0.001$) decreased the level of acid phosphatase in liver and kidney. MKEA was not able to produce a significant effect. There was significant difference between model group and control group in IL-1 and TNF- α level. After treatment with MKPE, MKEA and MKCF, the level of IL-1 and TNF- α in serum was lower than model group in MKPE and MKCF extracts. In the histological studies, the joints showed the destruction of inflamed joints and continued migration into the synovium and joint fluid of polymorphonuclear leukocytes, lymphocytes and monocytes/macrophages, all of which produce inflammatory cytokines. Thus, pharmacological inhibition of this leukocyte migration and accumulation in arthritis may have beneficial effects for joint preservation. In this study, histological interpretation supported that the administration of MKPE, MKCF (dose100mg/kg body weight) may be protective by decreasing the leukocyte migration. The alcohol extract of roots have shown very significant total phenolic content, total flavonoids content and free radical scavenging activity by DPPH and NO methods.

The pharmacognostical investigations i.e. morphological study of the *Ficus lacor* aerial root of the plant was performed. The aerial roots are typical roots and in transverse section it shows the features of a dicot root. The microscopy of the powder revealed the presence of annular xylem vessel, lignified fibre, parenchymatous cell and cork cells. Total ash, acid insoluble ash, water insoluble ash and sulphated ash were 14.15%, 8.57%, 10.75%, 6.00% respectively. The extractive values i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract were 5.7%, 10%, 5.5%, 4.5%, 10.5%. The fiber content was 9.45%. The plant can be used as bitter as its bitterness was found to be 1.9 unit/g. The foaming index was 124.6. The plant possesses haemolytic activity. The tannin content was 22. The alcoholic and aqueous extracts were screened for presence of amino acid and carbohydrates. The extracts showed the presence of three amino acid viz. alanine, methionine, ornithine and tyrosine and three carbohydrates i.e. galactose, lactose and sucrose. The

preliminary phytochemical screening of Pet. Ether extract, Ethyl acetate extract, Chloroform extract, Ethanol extract, Aqueous extract was performed. The presence of flavonoids, carbohydrates, saponins, phenolic compounds, and sterol in various extracts were observed. The three triterpenoidal phytoconstituents isolated (i.e. β -amyrin palmitate, β -amyrin stearate and Myristyl tetradecanoate) using different solvent in column chromatography. These compounds are first time reported in this plant. FLPE, FLEA, FLCF and FLET extracts from *Ficus lacor* aerial roots, both FLPE, FLET and FLTP-2 were found to be significant in reducing edema as similar to standard drug. Standard drugs produced more significant inhibition of edema. FLPE, FLET and FLTP-2 showed statistically significant inhibition of arthritic lesions ($p<0.05$) from day 16, ($p<0.01$) from day 20 and ($p<0.001$) from day 21 onwards. The extracts administered in higher doses reduced the lesions to a greater extent showing a dose dependent decrease in lesions. The terpenoidal fraction (FLTP-2) showed maximum inhibition as compared with control groups. The extracts FLPE and FLET showed significant increase in body weight ($p<0.001$) as compared to arthritic control group and increase in liver weight ($p<0.01$), decrease in liver weight ($p<0.001$) and increase in spleen weight ($p<0.001$) in arthritis control. FLEA did not show any significant result in body and organ weight estimation. The extracts FLPE and FLET showed significant decrease in WBC count ($p<0.001$), increase in hemoglobin contents and RBC counts as compared to control group. The extracts FLPE and FLET showed significantly ($p<0.001$) decreased the level of acid phosphatase in blood, whereas they also significantly ($p<0.001$) decreased the level of acid phosphatase in liver and kidney. FLEA and FLCF were not able to produce a significant effect. In the histological studies, interpretation supported that the administration of FLPE and FLET (dose 100mg/kg body weight) may be protective by decreasing the leukocyte migration. The alcohol extract of *Ficus lacor* aerial roots have shown very significant total phenolic content, total flavonoids content and free radical scavenging activity by DPPH and NO methods.

On the basis of above studies, it may be concluded that both plants are used empirically for the treatment of inflammation and arthritis in folk medicine. There is no data in literature about pharmacognostical, phytochemical and pharmacological potential of plants fractions.

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List of Abbreviations

AIA	Adjuvant induced arthritis
AMA	American Medical Association
BAW	Butanol: Acetic acid: Water
BHT	Butylated Hydroxy toluene
CA	Chromosomal aberrations
CAM	Cellular Adhesion molecule
CD 31	Cluster of differentiation molecules
CFA	Conjoint Freud's adjuvant media
CHF	Congestive heart failure
CIE	Carrageenan induced edema
CNS	Central Nervous system
COX	CYClooxygenase enzyme
COX-2	Cyclooxygenase-2
CS	Chondroitin sulphate
DJD	Degenerative joint disease
DJD	Degenerative joint disease
DMARD	Disease-Modifying Anti-Rheumatic Drugs
DMSO	Dimethyl Sulphoxide
DPPH	Diphenyl- 2-Picrylhydrazyl
E	Epidermal cells
ED ₅₀	Effective dose 50
EDTA	Ethylene diamine tetra acetic acid
ESR	Estimation of Sediment rate
FC	Ferric chloride/ Folins Ciocalteau reagent test for phenols
FEV	Forced expiratory volume
FIE	Formaldehyde/ Formalin induced edema
FL	<i>Ficus lacor</i>
FLCF	<i>Ficus lacor</i> Chloroform
FLEA	<i>Ficus lacor</i> Ethyl Acetate
FLET	<i>Ficus lacor</i> Ethanol
FLPE	<i>Ficus lacor</i> Petroleum Ether
GAG	Glycosaminoglycans
GIT	Gastrointestinal tract

HA	Hyaluronic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HNMR	Proton NMR spectra
ICAM	Intercellular adhesion molecule
IFN $\hat{\Upsilon}$	Interferon $\hat{\Upsilon}$
IL 8	Interleukin 8
IL-1	Interleukin 1
LAM	Leukocyte adhesion molecule
LD ₅₀	Lethal Doses 50
LOX	Lipoxygenase enzyme
LTA ₄	Leukotriene A ₄
LTB ₄	Leukotriene B ₄
LTC ₄	Leukotriene C ₄
LTD ₄	Leukotriene D ₄
LTE ₄	Leukotriene E ₄
M.E.V.	Mean Edema Volume
MAC -1	Integrin receptor for ICAM -1
MHC	Major histocompatibility complex
MK	<i>Murraya Koenigii</i>
MKCF	<i>Murraya Koenigii</i> Chloroform
MKEA	<i>Murraya Koenigii</i> Ethyl acetate
MKPE	<i>Murraya Koenigii</i> Petroleum ether
MS	Mass spectra
MSM	Methylsulphonylmethane
NO	Nitric oxide
NSAIDs	Non Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
P.I.	Percentage inhibition
PAF	Plasminogen activating factor
PECAM	Platelet endothelial cell adhesion molecule
PGD ₂	Prostaglandin D ₂
PGE ₂	Prostaglandin E ₂
PGF ₂	Prostaglandin F ₂
PGG ₂	Prostaglandin G ₂

PGI ₂	Prostaglandin I ₂
PMN	Polymorphonuclear leucocytes
RA	Rheumatoid arthritis
RBC	Red Blood Cell
ROS	Reactive oxygen species
S.E.M.	Standard Error of Mean
SSZ	Sulphasalazine
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TNF- α	Tumor Necrosis Factor
TPA	12-O-tetradecanoylphorbol-13 acetate
TXA ₂	Thromboxane A ₂
UV	Ultraviolet light
VCAM	Vascular cell adhesion molecule
VEGF	Vasculoendothelial growth factor
VLA-4	Integrin receptor for VCAM
VS	Vanillin Sulphuric acid reagent test
WBC	White blood cell

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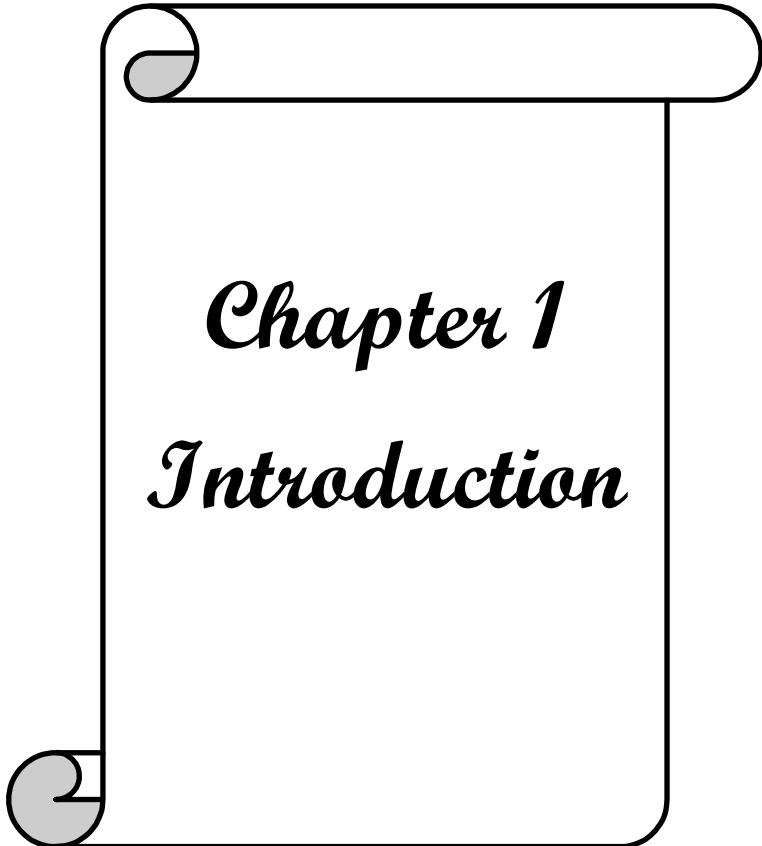
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Chapter 1

Introduction

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1.1 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 (Goldyne *et al.*, 1984). 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 (Woolf and Pfleger 2003). 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. Gains in life expectancy and aging populations are required to make the fourth leading cause of handicap by the year 2020 (Gautam *et al.*, 2009).

1.1.1 PATHOPHYSIOLOGY:

All inflammatory diseases have almost a common pathway of generation of disease (Fig 1.2), which involves generation of various inflammatory mediators at various stages due to initial stimulation by one or various etiological factors which may be an infection, an injury or even an allergic stimulus. The etiological agent causes increased vascular permeability after initial vasodilation and increased blood flow in the area due to release of various substances including Histamine from the mast cells in the areas. The increase in vascular permeability may be due to formation of endothelial gaps under the influence of Histamine, Leucotrienes, Bradykinins or Substance P (Majno and Palade 1961), or it may also be because of transcytosis which is due to intracellular formation of vesiculovacuolar organelles across the endothelial cells under the influence of VEGF and other factors. These vesiculovacuolar organelles act as channels across the endothelial cells increasing vascular permeability. It may also be because of endothelial retraction or cytoskeletal

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reorganization which creates gaps in between the endothelial cells under the influence of TNF, IL-1, IFN- γ (Feng *et al.*, 1996). It may also be because of leucocytes mediated lysosomal and proteolytic injury (Cotran and Brisco 1997).

Due to increased permeability and increased vascular blood flow, protein rich plasma starts exuding into the intracellular spaces. Simultaneously, accumulation of RBCs in the centre of blood vessels and marginalization of leucocytes to the periphery starts, where the leucocytes then show adhesion to the capillary walls with the help of receptors on the endothelial cells to which these cells bind through various adhesion molecules (Fig 1.1c). The major adhesion receptors and adhesion molecules are selectins (P-selectin and E-selectin receptors on the endothelial cells and LAM adhesion molecule on the leucocytes), Immunoglobulins (ICAM-1 and VCAM- 1 receptors on the endothelial cells), integrins (MAC-1 and VLA-4 on leucocytes binding to I-CAM and V-CAM respectively on the endothelial cells) and other adhesion molecules which include mucin like glycoproteins (Springer 1994). The adhesion process is stimulated by Histamine and PAF which induce reorientation of P-Selectins, cytokines (IL-1 etc) in which increase synthesis of adhesion molecules (E-Selectin, ICAM, VCAM) and then adhesion by various adhesion molecules and receptors.

The WBC then show transmigration or movement outside through the capillary walls into the interstitial spaces, the process being helped by various mediators particularly CD31, and PECAM (Muller 1996) (McEver 1997). The process is by pseudopod formation by the cells (Stossel 1993). After extravassation from the blood vessels into the tissue fluid, the leucocytes are further attracted to the site of inflammation by various chemotactic agents, which include endogenous substances, e.g. chemokines as well as bacterial products acting as chemotactic substances (Foxman *et al.*, 1997). The various endogenous substances include members of the complement system e.g., C₅A, products of lipoxygenase pathway, e.g., LTB₄ and cytokines e.g. IL₈. The binding with chemokines causes leucocytes to release more intracellular calcium by conversion of Phosphoinolpyruvate to Inositol pyrophosphate under the influence of phospholipase enzyme. This increased intracellular calcium stimulates contraction and pseudopod formation and movement towards the inflammatory area.

Subsequently, by the influence of chemokines, arachidonic acid metabolites are released by the leucocytes by the hydrolytic action of phospholipases on

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phospholipids and then converted to various eicosanoic acid derivatives (Fig 1.1). These include prostacyclins, prostaglandins and thromboxanes and finally various leucotriene derivatives. Side by side, there is release of lysosomal enzymes from the inflammatory cells by degranulation of lysosomal granules present within these cells. The leucocytes then start a process of phagocytosis (Fig 1.1 d) of exogenous inflammatory agents after opsonisation of these substances with opsonins e.g., C₃b, Fc, and Collectins (Klebanoff 1992). After opsonisation, engulfment of the inflammatory agents and simultaneous release of lysosomal hydrolytic enzymes takes place. These hydrolytic enzymes or hydrolases along with various oxidases cause hydrolytic and oxidative degradation of the causative agents into smaller components which are later disposed off. Simultaneously, the inflammatory injury may start resolving itself by new tissue formation and angiogenesis (if required), or it may start showing chronic inflammatory patterns and appearance of fibrotic scars characteristic of the type of tissues and the etiological causes. Various characteristic tissue changes may be observed particularly in synovial structures related to type of inflammation and etiological causes and are characterized as various forms of arthritis.

The formation of eicosanoids is critical to the progress of various stages of inflammation as these mediators affect every step of inflammation.

Formation of Eicosanoids (Dray 1995): The eicosanoid formation starts with the release of cellular phospholipids which are broken down by phospholipases and further by cyclooxygenases & lipoxygenases (Fig 1.1), to form:

- A. **Prostacyclins:** These compounds (PGI₂) have a variety of actions including vasodilation, hyperalgesia and decreasing platelet aggregation.
- B. **Prostaglandins:** These compounds also have a variety of actions including bronchospasm, vasodilation and hyperalgesia. PGG₂ is first formed which converts to PGH₂ which forms Prostacyclins, Thromboxanes and then PGD₂, PGE₂ and PGF_{βα} (cause vasodilation and potentiate edema). These compounds are further involved in the formation of Leukotrienes particularly LTA₄ etc.
- C. **Thromboxanes:** These compounds (TXA₂) form 5 Hydroperoxy acids which further play a role in inflammatory and repair process. They cause vasoconstriction and promote platelet aggregation.
- D. **Leukotrienes:** These are formed from arachidonic acid through the formation of 5 HPETE by 5-Lipoxygenase enzyme. LTA₄ is first formed which converts to

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LTB₄, LTC₄, LTD₄ and then LTE₄. All are vasoconstrictive and cause bronchospasm and increased vascular permeability.

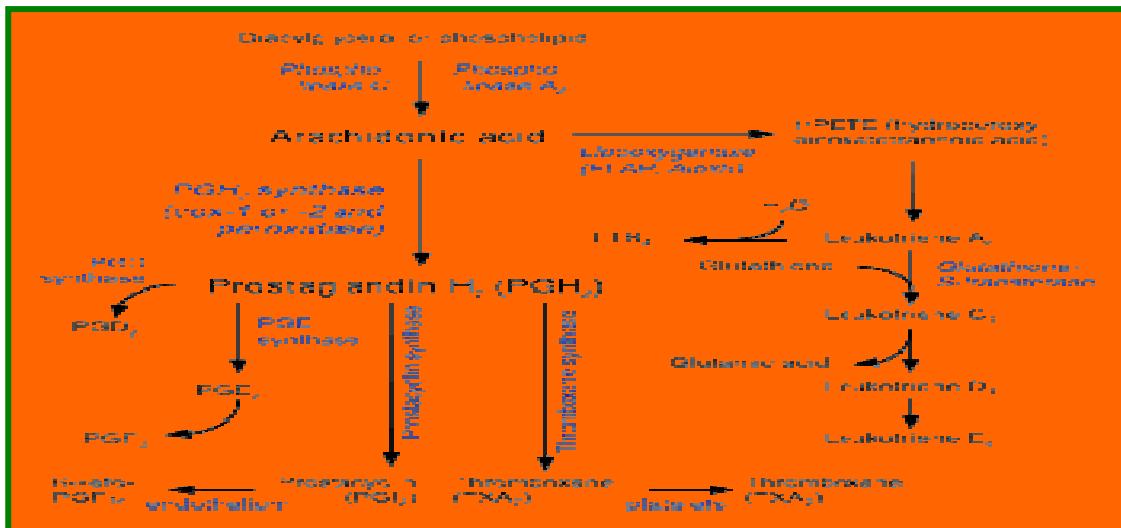
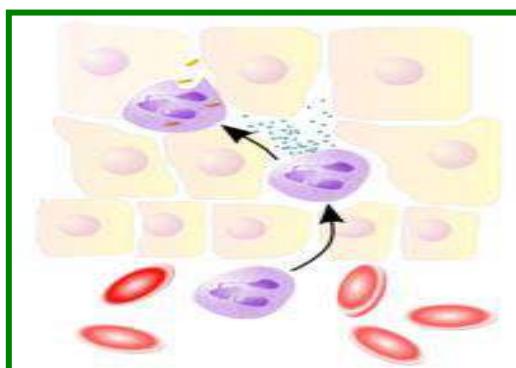


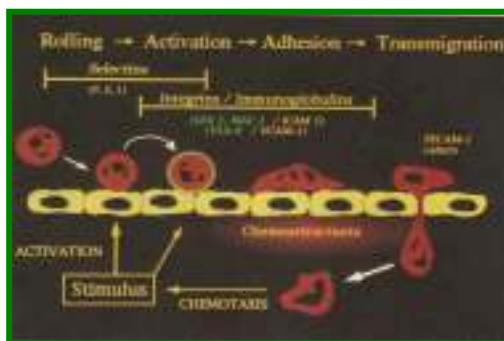
Fig 1.1: Formation of Arachidonic acid metabolites

The various mediators released, start a chain of reactions (Goetzl 1995) leading to the stages as in Fig 1.2:

- A. Stimulation by inflammatory agent/cause
- B. Increased vascular blood flow due to vasodilation, stasis, increased vascular permeability and plasma outflow.

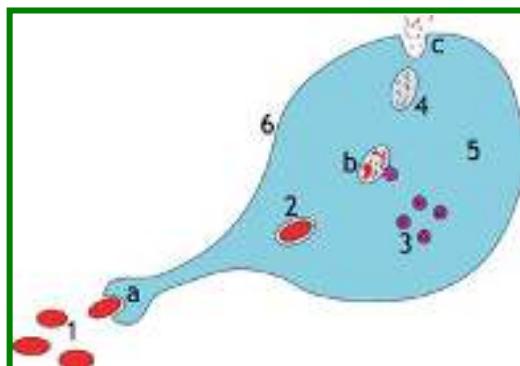


- C. Leucocyte adhesion & trans migration



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D. Chemotaxis, pseudopod formation & WBC movement, phagocytosis.



E. Release of lysosomal hydrolases and oxidases. Hydrolytic and oxidative degradation of inflammatory agents. Increased kinins, Histamines & other mediators (Mast cells of injured Tissues). Release of Arachidonic acid metabolites (Eicosanoids).

F. Vascular changes of Inflammation caused by Eicosanoids & G. End Products: Repair or Fibrosis

Fig 1.2: Pathophysiology of inflammation

Thus the main features of an inflammatory pathway are:

1. Increased vascular permeability.
2. Increased marginalization of leucocytes and other immune cells including monocytes, lymphocytes etc. within the blood vessels and increased transmigration of the white cells outside the vessels into the tissue space along with accumulation of fluids leading to inflammatory symptoms.
3. This is accompanied with lysosomal changes in the area leading to release of various enzymes, particularly hydrolases, which cause further inflammatory damage, particularly in the synovial spaces of the joints, affecting collagen and proteoglycans in the cartilage tissue.

1.1.2 The cellular components of the inflammatory process (Kumar *et al.*, 2001):

- a. These consist of polymorphonuclear cells, macrophages, and lymphocytes. Macrophages engulf and process antigens, which are then presented to T lymphocytes. The processed antigen is recognized by MHC proteins on the lymphocyte, which activates it to stimulate the production of T and B cells.

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- b. Activated T cells produce cytotoxins, which are directly toxic to tissues, and cytokines, which stimulate further activation of inflammatory processes and attract cells to areas of inflammation.
- c. Macrophages are stimulated to release prostaglandins and cytotoxins.
- d. Activated B cells produce plasma which forms antibodies. These antibodies in combination with complement system result in the accumulation of polymorphonuclear leukocytes (PMNs). These PMNs release cytotoxins, free oxygen radicals and hydroxyl radicals, which promote cellular damage. Autoimmunity also may play a role in this process.
- e. Lysosomal enzymes and other cytotoxic substances such as collagenases, other enzymes and free oxygen radicals may also be released from the inflammatory B cells (derived from bone marrow) or T cells derived from thymus tissue). T cells may be either T- helper or T- suppressor cells which modulate the inflammatory response. The majority of lymphocytes isolated from rheumatoid synovial tissue are T cell. It has been observed that patients with rheumatoid arthritis have an abnormal T to R- suppressor ratio compared with normal control.

The cellular components of acute and chronic inflammation are different and so also their morphological features.

1.1.3 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. Occasionally, there may be scarring and fibrosis or abscess formation or progression to chronic inflammation.

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

1. Persistent infections.
2. Prolonged exposure to potentially toxic agents.
3. 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 formation

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may also be a prominent feature in most cases of chronic inflammation (Majno and Joris 1996).

The monocytes responsible for chronic inflammation features later undergo modification into macrophages which release various enzymes which bring out the destruction, angiogenesis and fibrosis characteristic of the chronic inflammation, involving acid and neutral proteases, complement components and coagulation factors, eicosanoids and cytokines. The macrophages and lymphocytes may further enlarge and coalesce at the area of inflammation along with large number of plasma cells. Granulomas may also be formed by conversion of macrophages into large squamous cells but may not universally occur in all inflammations.

Both acute and chronic inflammation may further modify into:

1. Serous inflammation: Characterised by a watery serous fluid either from serum or mesothelial cells containing all inflammatory cellular discharges.
2. Fibrinous inflammation: Involving accumulation of extravascular fibrinous exudates along with other things.
3. Purulent inflammation: Characterised by large amount of purulent exudates or pus containing neutrophils, necrotic cells and edema fluid.
4. Ulceration: Characterised by necrosis of an epithelial surface which may further be eroded at various places. Along with neutrophilic infiltration, there may be fibroblastic proliferation and scarring.

Inflammation when present in association with the joints of the body presents itself in the form of various arthritic states which may have metabolic disbalance or immunological etiopathogenesis and may involve different set of joints to be classified as osteoarthritis, rheumatoid arthritis or other forms of arthritis. In most cases arthritic inflammation leads to degradative changes in the joints and associated cartilages.

1.2 Inflammation and joints: (Kumar *et al.*., 2001; Wilson Kathleen and Waugh 1999) Whatever may be the inflammatory pattern, the various joints in the body may show various morphological changes during an acute or chronic inflammation, if it is related to the musculoskeletal system, which may also lead to change in efficiency of movement. It is relevant here to discuss the morphology of a normal joint and then compare the changes induced by inflammation.

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1.2.1 The morphology of a normal joint (Fig 1.3): As a short figure, the very versatile diarthrodial joints of the body have homogeneous structures and parts including:

- a). The joint capsule or outer membrane: This encases the joint. The joint containers comprise of a thick stringy part, which is lined by a more slender subsynovium (lamina propria) and the synovium (synovial layer). The synovium or internal coating of the joint case comprises of cells, synoviocytes, which have both secretory and phagocytic capacities. Synovial covering cells incorporate hyaluronic acid (HA) that is secreted into the synovial liquid which involves the intra-articular space.
- b). Collateral ligaments, which are intra-capsular and give backing and steadiness to the joint (these work in conjunction with bracing muscle, other additional capsular ligaments, tendons and connective tissue).

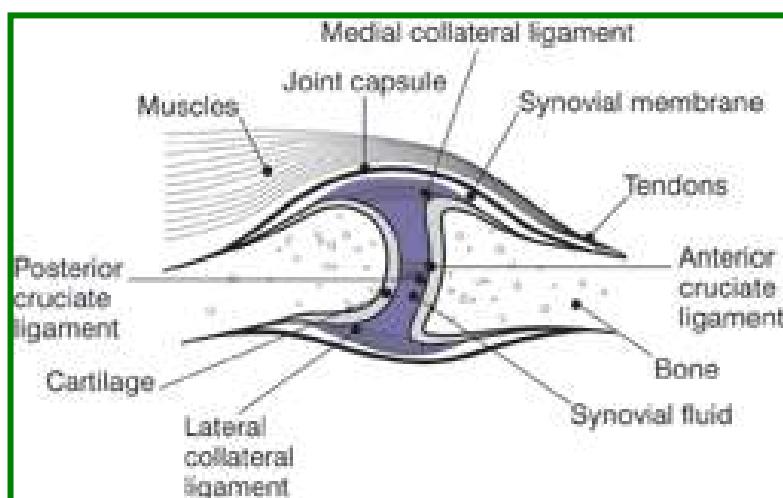


Fig 1.3: Morphology of a healthy Joint

- c. Articular cartilage, which cover the terminuses of the articulating bones inside the joints. Articular ligament is an exceptionally sorted out a vascular tissue made out of chondrocytes implanted inside an additional cell framework of collagens, proteoglycans and noncollagenous proteins and has a translucent or glasslike (hyaline) appearance because of its high dihydrogen monoxide content. Its essential capacity is to empower the smooth verbalization of joint surfaces, and to pad compressive, ductile and shearing powers. Hyaline ligament has one of the least coefficients of friction known for any surface to surface contact.

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- d. Subchondral bone, which gives basic backing to the overlying articular cartilage.
- e. The synovial fluid, which greases up and lubricates the joint surfaces. Synovial liquid is basically a ultrafiltrate of plasma containing hyaluronan (a non-sulfated glycosaminoglycan penniless of a protein center) discharged by the synovium. Synovial liquid is ordinarily exceedingly thick because of its hyaluronan content and assumes a significant part in keeping up salubrious ligament and defending the joint surface.

Hyaluronan is often known as hyaluronic acid (HA). This considerable glycosaminoglycan (GAG) is a basic piece of both synovial liquid and articular ligament. Inside the articular ligament, hyaluronan gives viscoelastic properties authorizing simplicity of kinematics between restricting surfaces and increasing compressive resistance. Inside the synovium, hyaluronic acid, as a part of synovial liquid, gives an effective hindrance directing the prelude of plasma parts. Under everyday conditions, the body will combine sufficient measures of base segments to keep up and become salubrious articular ligament, while encircling the engenderment and surrender of ruinous proteinases, incendiary arbiters and catabolic enzymes.

Composition of articular cartilage:

1. Around 70% water (up to around 80% water in neonatal creatures). (Harris 1989)
2. Collagens. The greater part of the collagen found in articular ligament is sort II collagen which gives malleable energy to the ligament. This diverse protein gives flexibility and the auxiliary structure of the ligament framework.
3. Proteoglycans, the other significant strong part of the articular ligament network comprise of one or more glycosaminoglycan affixes covalently clung to a protein center. Because of their thick negative particle content, these atoms have the capacity to polarize and hold dihydrogen monoxide inside the ligament arrangement particularly for grease. Proteoglycans give the novel mechanical properties to flexibility and instauration under compressive powers. The major proteoglycans found in ligament are Chondroitin sulfate, Dermatan sulfate, Keratan sulfate, and Hyaluronan. More early names for proteoglycans at some point allude capacity of the center protein inside the particle, e.g., aggrecan (found in chondroitin sulfate and keratin sulfate), a cosmically gigantic proteoglycan total with hyaluronan, or allude area, e.g. decorin (dermatan sulfate), which enhances sort I collagen fibrils, or to

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essential structure, biglycan which has two glysoaminoglycan chains. Chondrocytes are dynamic cells inside the ligament lattice, which produce early collagen and proteoglycan particles while discharging catalysts, which benefit in deliberation of harmed ligament and proteoglycans.

1.2.2 Inflammatory injury to synovial cartilage (Graziano and Bell 1985):

Under unremarkable conditions, the body keeps up the synovial joint in a condition of homeostasis through a mixed bag of involute hormonal and mechanical criticism systems. Two sorts of affront or damage can annoy the fragile homeostatic offset. Emphasized injury or anxiety (moderate incessant affront) to the joint amid ordinary utilization, e.g., athletic preparing or execution, is regularly the prompting reason for joint irritation and loss of homeostasis. At first, such stretch results in just delicate tissue aggravation as synovitis or capsulitis (e.g., traumatic synovitis). Ligament harm could possibly at first be introduce in the early phases of anxiety related damage or irritation. Intense aggravations frequently may change over to perpetual aggravation which may harm the synovial structures and induce sundry ligament conditions which are connected with astringent irritation and unbearable torment in downright joints of the body. The surrender of provocative middle people into the joint, for example, prostaglandis, cytokines, lysosomal catalysts and free radicals can prompt harm of articular ligament and can bring about ligament debasement and can prompt advancement of degenerative joint ailment (DJD) (Graziano and Bell 1985). Because of its avascular nature, articular ligament has extremely encircled limit for repair and reviving all alone. As cartilage damage continues, bone ends become exposed and stressed, then develop cysts and spurs (osteophytes) causing the bones to rub painfully together. Tissues of the joint capsules also become inflamed and damaged. Pain leads the joint's owner to reduce activity, which leads to weakened muscles, increased wear, which creates more damage and more pain. The end results of the chronic inflammatory changes in a synovial tissue are variable and include:

1. Loss of cartilage which may result in a loss of the synovial space.
2. The formation of chronic granulation or scar which can lead to loss of joint motion or bony fusion or bony ankylosis.

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3. Laxity of tendon structures which can result in decreased support to the affected joint leading to instability or deformation. Tendon contractures may also occur leading to chronic deformity (Harris 1989; Synderman 1986).

Focal articular cartilage defects, often found in puerile adults, have been increasingly apperceived as a cause of pain and functional quandaries. There is more clinical confirmation that full thickness articular ligament imperfections sustain to advance and disintegrate, yet at a moderate rate. Early determination and treatment is prescribed preceding the advancement of more propelled osteoarthritis. The ligament tissue is difficult to work with and aggravation and wounds to joint surface, whether traumatic or degenerative, are unforgiving, and the movement to sundry joint stages e.g. osteoarthritis is here and there too ease back to identify making hindrance of irritation a weighty prophylactic methodology.

- 1. 3 Arthritis: Features and types** (Zimmerman 1989; Hamerman 1989; Tripple 1990; Brudveld *et al.*, 1997; Harris 1990).

Joint inflammation is a state of aggravation with torment in one or more joints. Contingent upon the aggravation and torment example and the sort of joints included, it has been arranged into the accompanying sorts: of lost tissues by cell multiplication and blend of early intracellular network Infelicitously, restored articular ligament for the most part neglects to recreate the structure, piece, and capacity of ordinary articular ligament. Counterfeit repair now and then uses lavage. Lavage may relieve the indications and augmentation the versatility and firmness of articular ligament by transmuting the ionic environment inside the synovial liquid. In any case, the lavage gives just transient symptomatic vindication without amendment of basic pathology. Regeneration in this setting alludes to the development of a completely nascent articulating surface that basically copies the unblemished articular ligament. Along these lines, all the better we can do at present is blockers - anakinra; Anti-B cell (CD20) immunizer - rituximab (Rituxan); Blockers of T cell initiation - abatacept (Orencia). DMARDs have to incite strong reductions and postpone or end malady movement. Specifically they deflect bone and joint harm from happening optional to the uncontrolled irritation. This is noteworthy thusly harm is generally irreversible. Mitigating operators and analgesics change torment and firmness yet don't forestall joint harm or moderate the illness movement. There may be different reasons why beginning DMARDs early is benevolent and also hindrance of basic joint harm.

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In the early phase of the infection, the joints are progressively invaded by cells of the safe framework that sign to each other and are thought to secure maintaining toward oneself constant aggravation. Interfering with this methodology as right on time as could be allowed with an adequate DMARD, (for example, methotrexate) seems to improve the result from the RA for a considerable length of time subsequently. Deferring treatment for as tiny as a couple of months after the onset of side effects can bring about more awful results in the long haul. There is in this manner impressive enthusiasm for making the most viable treatment in patients with joint inflammation, when they are most receptive to treatment and have the most to pick up.

1.3.1 Osteoarthritis:

It is a ligament condition pervasive among more seasoned individuals. Now and then it is called degenerative joint illness or osteoarthritis. In osteoarthritis, the surface layer of ligament splits down and wears away. This authorizations bones under the ligament to wind up smooth surfaced and rub together, bringing about torment, swelling, and loss of development of the joint (Fig 1.5). After some time, the joint may lose its ordinary shape. Withal, moment stores of bone called osteophytes or bone goads – may become on the edges of the joint. Bits of bone or ligament can sever and skim inside the joint space. This causes more agony and harm. As the bone surfaces get to be less very much bulwarked via ligament, the patient encounters torment upon weight bearing, including ambulating and standing. Because of decremented development due to the agony, provincial muscles may decay, and ligaments may get to be more remiss. OA represents 25% of visits to essential consideration medicos, and a moiety of all NSAID (Non-Steroidal Anti-Inflammatory Drugs) medicines. It is evaluated that 80% of the populace have radiographic proof of OA by age 65, yet just 60% of those get to be symptomatic (Green 1991). The principle indication is interminable agony, bringing about loss of versatility and regularly solidness. "Agony" is for the most part depicted as a sharp throb or a blazing sensation in the related muscles and tendons and is because of initiation of nociceptive nerve terminuses by synthetic aggravations (Zimmerman 1989). OA can bring about a crackling commotion (called "crepitus") when the influenced joint is moved or physically reached, and patients may experience muscle fit and withdrawals in the tendons. Occasionally, the joints might withal be loaded with liquid. Sultry climate builds the torment in numerous patients. OA generally influences the hand,

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feet, spine, and the cosmically huge weight-bearing joints, for example, the hips and knees, yet in principle, any joint in the body can be influenced. As OA advances, the influenced joints seem all the more massively monster, are firm and difficult, and traditionally feel more terrible, the more they are used for the duration of the day, along these lines recognizing it from rheumatoid joint inflammation.

In more small joints, e.g., at the fingers, hard growths, called Heberden's hubs (on the distal interphalangeal joints) and/or Bouchard's hubs (on the proximal interphalangeal joints), may shape, yet despite the fact that they are not compulsorily excruciating, they do restrain the kineticism of the fingers altogether. OA at the toes prompts the arrangement of bunions, rendering them red or swollen (Anonymus, 1998).

The eteopathogenesis proposes wear and tear, biomechanical, biochemical, provocative, hereditary and immunological elements as in charge of the advancements (Hamerman 1989; Tripple 1990). The biochemical changes in the ligament amid osteoarthritis are described by expansion in Chondroitin 4 sulfate to chondroitin 6 sulfate degrees, increment in proteases, diminish in glycosaminoglycans and reduction in proteoglycan conglomeration (Ratcliffe 1990). The fundamental morphological changes incorporate harm of collagen filaments and abatement in proteoglycan substance and increment in PG action prompting expanded hydration and development. The expanded hyaline water reasons change in Chondroitin 4 sulfate to Chondroitin 6 sulfate degree bringing on disbalance between collagen framework amalgamation and hydration. This prompts net disintegration with loss of PG totals of the ligament Osteoarthritis for the most part happen in the hands , spine (neck and lower back), knees, and hips (Fig 1.4).



Fig 1.4: Comparison of a healthy joint with severe osteoarthritis and Rheumotoid arthritis

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1.3.2 Rheumatoid arthritis (RA):

It is traditionally viewed as a chronic, inflammatory immune system issue that causes the insusceptible framework to assault the joints (Brudveld *et al.*, 1997). It is a weakening and difficult incendiary condition, which can prompt generous loss of versatility because of agony, symmetric ligament design in joints (begining at a more youthful age than osteoarthritis) and joint destruction alongside sacred manifestations of anorexia, despairing, weight reduction and exhaustion (Rodnan 1988) as indicated in fig. 1.4. RA is a systemic ailment, frequently influencing additional articular tissues all through the body including the skin, veins, heart, lungs, and muscles (Harris 1990). The reason for RA is still obscure right up 'til the present time, however has long been suspected to be irresistible. It could be because of victuals unfavorable susceptibilities or outer organic entities. Mycoplasma, Erysipelothrix, Epstein-Barr infection, parvovirus B19 and rubella have been suspected yet never sustained in epidemiological studies. As in other immune system maladies, the "misinterpret personality" hypothesis proposes that a culpable organic entity causes an insusceptible replication that abandons antibodies that are cement to that life form (Harris 1989). The antibodies are not sufficiently solid, however. They begin a safe assault against, for this situation, the synovium, in light of the fact that some particle in the synovium "looks homogeneous to" an atom on the culpable organic entity that induced the starting insusceptible response - this wonder being called as atomic mimicry or autoimmunity. At the same time physical and passionate impacts, hereditary attributes, anxiety and infelicitous eating routine could assume a part in the ailment. The general occurrences can be outlined as antigen sensitisation of WBCs with MHC proteins which tie to T cells with CD-4 and afterward give up of cytokines which activate B cells to bring about pannus arrangement and joint harm by resistant multifaceted development through Rh component. The cytokines furthermore cause enactment of endothelial cells, surrender of incendiary cells and pannus development by PGE2 elastase and different chemicals. Immune system maladies oblige that the influenced individual have an imperfection in the capacity to separate self from peregrine particles. There are markers on numerous cells that present this perceiving toward oneself highlight. Notwithstanding, a few classes of markers assent for RA to transpire. 90% of patients with RA have the group of markers kenned as the HLA-DR-4/DR-1 bunch, though just 40% of unaffected controls do. Along these lines, in

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principle, RA obliges defenselessness to the infection through hereditary enrichment with unmitigated markers and an irresistible occasion that triggers an immune system replication. Customarily all RA patients ought to have a positive Rh variable (Rh+ ve) (Rodman and Schumacher 1988). Once set off, the resistant replication causes irritation of the synovium (Sakkas and Platsoneas 1995). Early and halfway subatomic middle people of irritation incorporate tumor corruption component alpha (TNF- α), interleukins IL-1, IL-6, IL-8 and IL-15, changing amplification variable beta, fibroblast amplification element and platelet-determined amplification component (Muller Ladner 1996). Current medicines of RA target these mediators. Once the incendiary response is built, the synovium thickens, the ligament and the basic bone start to crumble and confirmation of joint ravagement accumulates.

The theory that earlier contamination by microscopic organisms may be the activity element for the begin of the immune system response and anti-toxins ought to be consolidated into the treatment has been recommended by numerous studies.

Tetracycline anti-toxins have been utilized to treat rheumatoid joint pain, and change in indications is confirmation that the anti-toxin must be slaughtering a bacterium that brought on the joint inflammation. The tetracycline anti-microbials however furthermore "display immunomodulatory properties, which may contribute fundamentally to their healthy impacts in rheumatoid joint inflammation". As such, the same medication can both eliminate microscopic organisms and smother the insusceptible framework, and the last may be in charge of its advantages in rheumatoid joint inflammation. That verbally communicated, there are a great many archived instances of reduction of RA and other related auto-safe infections using anti-toxins, and numerous different reports from rheumatologists ecumenical that reasonable use of minocycline, at times blended with clindamycin, alongside eating regimen change, alimental supplements, and energetic activity, and sundry other resistant sustaining option treatments, for example, needle therapy, hyperbaric oxygen, and infra-red sauna, can retard or dispatch the sickness. Tumor putrefaction element inhibitors are a class of medications that uniquely decrease imperviousness to specific sorts of bacterial disease yet are extremely effectual in treating RA. Indeed, they are a standout amongst the most adequate medicines for rheumatoid joint inflammation in far reaching utilization. The microbes/anti-infection speculation consequently has next to no backing amongst old-line, moderate rheumatologists and

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analysts, however is optically perceived by more integrative medicos and parental figures as a segment of the web of components that incite the sickness. A few sizably voluminous, long haul mulls over in the most recent ten years have demonstrated that anti-toxins have a considerable part to play in the armamentarium of weapons against RA, and an advanced type of the anti-toxin convention has now been formally authorized as a DMARD (illness adjusting hostile to rheumatic medication) by the AMA and apperceived by the Arthritis Substratum.

1.3.3 Septic joint inflammation:

It is the expansion of microorganisms in joints and resultant inflammation. Microscopic organisms are either conveyed by the circulation system from an irresistible concentrate somewhere else or are presented by a skin injury that punctures the joint.

Septic joint inflammation ought to be suspected when one joint is influenced and the patient is hot. In seeding joint inflammation, different joints can be influenced in the meantime; this is particularly the situation when the contamination is created by staphylococcus or gonococcus microbs. Finding is by yearning (giving a turbid, non-thick liquid), Gram stain and society of fluid from the joint, and also indications in lab testing, (for example, a profoundly hoisted neutrophils, ESR or CRP).

1.4 Management of arthritic inflammation and degenerative damage:

Management of inflammation is of prime importance in treatment of various arthritic conditions apart from treating the underlying metabolic or immunological cause or infection, and requires judicious use of available anti-inflammatory agents for a suitable period. The degradative changes caused in the synovial cartilage due to arthritic inflammation also have to be taken care of.

1.4.1 Approaches to management of arthritic synovial damage and rebuilding (Arnold 1988; Reichenbach *et al.*, 2007):

In arthritic conditions, cells that make up the articular cartilage are damaged and don't repair themselves, setting off a chain reaction of joint destruction. The fraying cartilage creates friction and sheds debris into the synovial fluid, increasing inflammation that in turn increases cartilage damage.

Thus the articular cartilage has to be either:

- a. Repaired
- b. Regenerated, or

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- c. Prevented and protected from damage during chronic inflammatory phases.

Repair refers to the rejuvenating of injured tissues or supersession of damaged tissue and combination of incipient intracellular matrix. Infelicitously, reformed articular cartilage usually fails to imitate the composition, structure, and function of ordinary articular cartilage.

Artificial repair sometimes utilizes lavage. Lavage may mitigate the symptoms and increment the resiliency and stiffness of articular cartilage by transmuting the ionic environment within the synovial fluid. However, the lavage provide only short time indicative palliation without rectification of underlying pathology.

Regeneration in this perspective refers to the development of an entirely budding articulating surface that essentially duplicates the pristine articular cartilage. Therefore, the best we can do at present is to rehabilitate the chondral defect. Even the prevention of cartilage damage during inflammatory phases is a more appropriate option.

Thus, a prominent area of research in articular damage in osteoarthritis and similar conditions is prevention of cartilage damage and for this, agents acting at various stages during inflammatory joint degeneration can be screened. These may be:

1. Agents preventing initiation of inflammatory responses.
2. Agents preventing release of degradative lysosomal enzymes at the site of inflammation.
3. Other agents.

Thus the importance of an anti-inflammatory agent which also shows potential to prevent synovial damage is clearly understood in the search for new anti-inflammatory compounds. Under any type conditions, once compromise, the damage to articular cartilage is conventionally perpetual. In general, once damaged, therapy is mundanely directed at inhibiting or reducing joint inflammation, inhibiting the relinquishment of inflammatory mediators, abstraction of the inciting cause (e.g., the chip) and supersession of synovial fluid components. These quantifications are cumulated with a period of rest to sanction for rejuvenate and fibrocartilage evidence at the affected area. The extensive term medicinal use is directed at slow the succession of degenerative processes and controlling the clinical designations of DJD.

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1.4.2 Treatment and management of arthritic inflammation: (Arnold 1988; Rainer 1988; Hasler, 2006)

No significant progress has been achieved in getting a permanent cure once the inflammation has set in and initiated a process of articular damage (Huscoisson *et al.*, 1985). But there have been innumerable therapies for management of inflammatory and arthritic diseases once they have occurred (Dell 2004; William and Clegg 1990).

These include:

Management of pain and inflammation by anti-inflammatory and other drugs.

Management of immovability of joints at the required level by physiotherapy and other supportive measures.

Prevention of infection by therapeutic use of antibiotics where the immune response is supposed to be triggered off due to these infectious conditions. The health food for nutritional supplements of metabolic precursor to aid in the biosynthesis of proteoglycans, GAG's, hyaluronan, and collagen which are lost during the progression of the arthritic conditions.

Drug treatment of various arthritis states and management of pain and inflammation presently comprises of one or more of the following strategies:

I. Use of DMARDs (Disease modifying antirheumatic drugs): As rheumatoid arthritis has been proposed to be precipitated by an infectious attack leading to development of autoimmunity against the synovial components, many immunosuppressants are recommended by exhaustive studies as disease modifying agents (DMARDs) (Arnold 1988; Rainer 1988). These include Xenobiotics and Biological agents.

Xenobiotics include:

Azathioprine, Minocycline, Cyclosporine (cyclosporine A), D-penicillamine, Hydroxychloroquine , Leflunomide, Gold salts,Methotrexate, Sulfasalazine (SSZ).

Biological agents include : (TNF α) blockers - etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira); IL-1 blockers - anakinra; Anti-B cell (CD20) antibody - rituximab (Rituxan); Blockers of T cell activation - abatacept (Orencia).

DMARDs need to incite sturdy reductions and postpone or stop malady movement. Specifically they deflect bone and joint harm from happening auxiliary to the uncontrolled inflammation. This is considerable all things considered harm is usually irreversible. Calming operators and analgesics alter torment and firmness yet don't deter joint harm or moderate the malady movement. There may be different reasons

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why beginning DMARDs early is favorable and in addition hindrance of basic joint harm. In the early phase of the illness, the joints are progressively penetrated by cells of the invulnerable framework that sign to each other and are thought to make engendering toward oneself constant irritation. Interfering with this procedure as right on time as could reasonably be expected with an strong DMARD, (for example, methotrexate) seems to improve the result from the RA for quite a long time subsequently. Deferring treatment for as microscopic as a couple of months after the onset of manifestations can bring about more awful results in the long haul. There is in this manner impressive enthusiasm for building the most adequate treatment in patients with right on time joint inflammation, when they are most receptive to treatment and have the most to increase (Vital E 2005).

II. Use of anti-inflammatory agents (particularly NSAIDS), and corticosteroids and analgesics: There are different chemical classes of NSAIDs each with a specific mechanism of action and particular pharmacokinetics (Brooks 1991). These include agents which are non selective inhibitors of cyclooxygenase and selective Cyclooxygenase (COX-2) inhibitors:

I. Nonselective COX-2 inhibitors:

- A. Salicylates: Include Aspirin, Amoxiprin, Benorilate, Choline magnesium salicylate, Diflunisal, Faislamine, Methyl salicylate, Magnesium Salicylate, Salicyl salicylate (salsalate).
- B. Arylalkanoic acids: Include Diclofenac, Aceclofenac, Acemetacin, Bromfenac, Etodolac, Indometacin, Ketorolac, Nabumetone, Sulindac, Tolmetin
- C. 2-Arylpropionic acids (profens): Include Ibuprofen, Carprofen, Fenbufen, Fenoprofen, Flurbiprofen, Ketoprofen, Loxoprofen, Naproxen, Tiaprofenic acid, Suprofen
- D. N-Arylanthranilic acids (fenamic acids): Include Mefenamic acid, Meclofenamic acid, alkalonones, nabumetone
- E. Pyrazolidine derivatives: include Phenylbutazone, Azapropazone, Metamizole, and Oxyphenbutazone.
- F. Oxicams: Include Piroxicam, Lornoxicam, Meloxicam, Tenoxicam
- G. Indole acetic acid derivatives

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II. Selective Cyclooxygenase (COX-2) Inhibitors:

Include diaryl substituted pyrazole derivatives, e.g., Celecoxib, Etoricoxib, Lumiracoxib, Parecoxib, Diaryl substituted furanones e.g., Rofecoxib (withdrawn from market 2004), Valdecoxib (withdrawn from market 2005), Sulphonanilides, e.g., Nimesulide and others, e.g., Licofelone, Omega-3 Fatty Acids etc.

Combination of NSAIDs with other NSAIDs or aspirin provides no added benefit but increases the toxic effects with GIT complaints being the most common and accounting for many treatment failures (Barrier 1989).

III. Use of antibiotics in some cases.

IV. Use of nutritional supplements to control and prevent damage to synovial cartilage during arthritis:

Oral nutritional supplements containing metabolic precursors for the synthesis of articular cartilage which consist of:

1. Chondroitin sulfate, Glycosaminoglycan Polysaccharide, part of articular ligament containing an amino sugar.
2. Glucosamine
3. Methylsulfonylmethane (MSM).
4. Sodium hyaluronate (hyaluronan) etc.

Chondroitin sulfate is separated into sulfate disaccharides and N-Acetyl galactosamine. D-Glucuronic corrosive is a significant substrate containing one a large portion of the hyaluronan atom, the other being N-Acetyl D-glucosamine. Chondroitin sulfate, stay as CS4 and CS6 inside the body, are to be crucial glycosaminoglycans which tie water to the articular ligament grid and are critical for the arrangement of proteoglycans. Chondroitin sulfate, when known as an outer source acts to energize the creation of Hyaluronic corrosive, hinder degenerative catalysts discharged by the chondrocytes, and synoviocytes, and support in declining the vicinity of provocative go betweens inside the synovial liquid (Reichenbach *et al.*, 2007). The N-acetyl D glucosamine give in the exogenous syntheses builds the generation of synoviocytes and chondrocytes, taking after accessibility of hyaluronan inside the joint and ligament framework by the straight in situ incorporation of its prime substrates galactosamine and N-Acetyl D-glucosamine One more oral supplement, glucosamine, as glucosamine 5-phosphate, is clearly happening inside the body and it is a segment in the biosynthesis of glycosaminoglycans, proteoglycans,

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hyaluronan, and collagen. Glucosamine is display in exogenous structures, glucosamine sulfate sodium, glucosamine hydrochloride and N-Acetyl D-Glucosamine. These structures are accounted for to be orally bioavailable in vertebrates. Exogenous glucosamine is accepted to permit the body to surpass the regular rate-constraining limits whereby glucosamine turns into the stimulant in the generation of proteoglycans and GAGs Poolsup *et al.*, 2005). Exogenous oral glucosamine is likewise accepted to invigorate chondrocytes to create more collagen and improve articular digestion system (McAlindon *et al.*, 2004). Methylsulfonylmethane likewise utilized as a part of oral supplements, it is a key piece of hemoglobin and body tissue, and important for the blend of uniting tissues, collagen and the critical amino acids like methionine and cysteine. Sodium ascorbate is an oral supplement that is needed for collagen union and supports in the body's capacity to use manganese. In principle, oral supplementation and taking after biosynthesis of the metabolic forerunners support in the generation of new articular ligament even as helping in amendable the harming impacts of damaging catalysts. The chondroitin sulfate of the creations gave thus is ideally in arrangement or suspension with N-acetyl D-glucosamine furthermore, hyaluronan. Notwithstanding that impact, the joining of chondroitin sulfate into the creations gave thus serves to ease off the provocative procedure, acting straightforwardly on the chemicals, incendiary methodology and incendiary arbiters which are discharged when irritation is available.

The sodium hyaluronate (hyaluronan) when given as outside supplementation serves to cover the surface of the joint container and articular ligament with a slim covering to support in imperviousness to ligament pressure while holding flexibility; it likewise cooperates with proteoglycans to shape a stabile total, and giving greasing up properties. Hyaluronan likewise straightforwardly supports in the expulsion of waste items from the joint capsule and goes about as an inhibitor of provocative go betweens. The exogenous Hyaluronan replaces drained endogenous HA and greases up and covers sound and additionally harmed articular ligament furthermore decreases aggravation of synovial films to treat and/ or anticipate various NSAIDs have been investigated for their role in proteoglycan biosynthesis and chondroprotective activity and their effects are not fully established (Burkhardt and Ghosh 1987; Ghosh 1987).

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1.5 Mechanism of action of various synthetic & semisynthetic anti-inflammatory drugs.

Various anti-inflammatory agents act by various mechanisms (Brooks and Day 1991; Abramson and Weisman 1989; Craig and Buchnan 1980) such as:

1. Irreversible time dependent inactivation of cyclooxygenase enzymes (Aspirin) (Roth and Siok 1978).
2. Rapid reversible competitive inhibition of cyclooxygenase enzyme (Ibuprofen) (Flower and Vane 1974)
3. Rapid reversible non competitive inhibition of enzyme.
4. Scavenging of oxygen free radicals, & decreasing tissue damage.
5. Interfering with binding of chemical mediators with chemotactic peptides from bacteria.
6. Interference with synthesis and action of Cytokines (IL₁ or TNF α), e.g., adrenocorticosteroids.
7. Cysteinyl Leukotriene receptor inhibition, e.g., Monteleucast, Zafirleucast etc.
8. 5-Lipoxygenase inhibition. E.g., Zileuton.
9. Platelet inhibition, e.g. aspirin, ketorolac etc.
10. Stabilisation of lysosomal membranes, e.g., Ketoprofen.
11. Decrease in intracellular accumulation of free Arachidonates in leucocytes by altering release or uptake of fatty acids, e.g., Diclofenac.
12. Selective COX-2 inhibition, e.g., Rafecoxib, Celecoxib etc.
13. Inhibition of transmembrane ion fluxes, inhibition of enzyme proteoglycan synthase and modification of immune responses are some other mechanisms.

As can be seen most of the above approaches are based on inhibition of formation of arachidonic acid and Prostaglandins from phospholipids precursors as arachidonic acid and prostaglandins are important mediators for causing most of the physiological and morphological changes as observed during inflammation. Other approaches which may be relevant (William and Clegg 1990; Hart and Huskisson 1984) are:

1. Decrease in vascular permeability by inhibition of release of vasodilating inflammatory mediators, like Histamine etc.
2. Inhibition of migration and chemotaxis of leucocytes to the site of inflammation, e.g., Hydroxychloroquin.

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3. Inhibition of the release of degradative lysosomal enzymes which bring about degradation of the synovial tissues and thus preventing the progression of arthritic changes.
4. Inhibition of generation of superoxide radicals by phagocytes, e.g., by steroids.
5. Inhibition of liposomal enzyme release from polymorphonuclear leucocytes.
6. Decrease in lymphokine production.
7. Inhibition or decrease of the immune response which is part of the changes brought about in condition such as Rheumatoid arthritis, e.g. decrease in T-lymphocyte function by Penicillamine. Use of Corticosteroids (Myles 1985) and other newer antiinflammatory disease modifying antirheumatic drugs (DMARD) drugs like Methotrexate (Brick 1989) etc., is a part of this strategy which can inhibit the inflammatory responses as well as the changes due to synovial damage observed during various types of arthritis. Very few of the in use anti-inflammatory agents combine their anti-inflammatory activity with disease modifying activity and synovial damage inhibiting activity. Natural products, including crude drugs and phytoconstituents, either alone or in combination, are supposed to offer multiple advantages in management of inflammatory conditions. Therefore the interest in screening natural products as potential source for anti-inflammatory lead compounds is logical.

1.6 Natural medicine as anti-inflammatory and antiarthritic drugs:

Despite rheumatism and inflammatory diseases being one of the oldest known diseases of mankind affecting large population of the world, no substantial progress has been made in achieving permanent cure. With screening and development of synthetic anti-inflammatory drugs posing unending problems, there is much hope of finding active anti-inflammatory and arthritic compounds from indigenous plants as they are still used in therapeutics despite the progress made in conventional chemistry and pharmacology for producing effective drugs (Handa 1992). Major drawback with synthetic anti-inflammatory substances is non selective inhibition of COX I & COX II due to which these drugs also inhibit biosynthesis of prostaglandins at other areas within the body e.g., GIT, uterus etc, where their presence plays crucial role in proper physiological functioning. Thus most of the synthetic antiinflammatory drugs have a potential for causing gastric ulcer by decreasing prostaglandin synthesis in the gastric mucosa which causes increased gastric acid secretion potentiating their ulcerogenic

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potential (Barrier 1989). Immunosuppressive DMARDs to produce some or the other side effects associated with suppression of immunity. Further, on prolonged use in high doses, these drugs can cause renal complications, renal insufficiency, and hyperkalemia particularly in CHF, cirrhotic and ascitic patients (Clive and Stoff 1984; Garella and Matarese 1984).

Crude drugs and related natural products are therefore considered as a potential alternatives exhibiting antiinflammatory and antiarthritic activity with minimum or no ulcerogenic potential and are therefore important sources for discovery of lead compounds showing such activity. Many natural products are reported to increase PGE₁ and Mucin content in GIT mucosa and decrease gastric acid secretion apart from their anti-inflammatory activity. Further, many of the crude drugs and their products have constituents which have antioxidant activity and scavange free radicals which cause propagation of inflammatory responses and cause cartilage damage. Many plants contain flavonoids and coumarins in different parts which have good scavenging potential. Tylophorine from *Tylophora indica* and various other constituents from other plants have also been shown to be good immunosuppressants. Some plant products, for example lupeol and its derivative lupeol linoleate have also been shown to inhibit release of lysosomal enzymes which are responsible for cellular damage associated with inflammation in arthritis which leads to synovial cartilage damage (Geetha 1999). Thus apart from anti-inflammatory activity, natural products offer multiple activity profiles with lesser adverse effects.

Various plant drugs may produce their anti-inflammatory actions by various proposed mechanisms as follows:

1. Inhibition of cyclooxygenase enzyme (COX I and II).
2. Inhibition of Leucocyte migration to the site of inflammation.
3. Inhibition of Arachidonic acid synthesis.
4. Inhibition of release of histamine from mast cells.

A review of earlier works on plants with anti-inflammatory activity shows that a number of plants with diverse constituents show anti-inflammatory activity against inflammation induced by various phlogistic agents.

1.6.1 Plants reported for anti-inflammatory activity:

From time immemorial plants are used as medicine around the world and plant based medicine has been the mainstay of traditional societies in dealing with health

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problems (Pramila, 2006). The World Health Organization (WHO) has estimated that 80% of the earth's (6 billion) inhabitants rely upon traditional medicine for their primary health care needs and major part of this therapy involves the use of plant extracts or their active principles (Farnsworth, 1994; Kurain, 1995). The fields of Botany, Pharmacognosy, Phytochemistry, Biochemistry, Ethnopharmacology and Toxicology are integral parts of herbal medicine (Singh, 2007). Ceaseless use of natural prescription by an extensive extent of the populace in the creating nations is generally because of the high cost of Western pharmaceuticals and medicinal services. Also natural meds are more worthy in these nations from their social and profound perspectives (Cunningham, 1994). In India 60-70% patient living in rural areas are dependent on herbal medicine for their day to day disease (Singh, 2007). Number of diseases is cured by herbal drugs. The arthritis and inflammation also cured by herbal medicines.

Table 1.1: Various plants are being effectively used as a anti-arthritis and anti-inflammatory agents.

S. No.	Common name	Botanical Name	Parts Used/ Extract	References
1.	Needle Bush	<i>Acacia farnesiana</i> Leguminaceae	small petals	(Duke, 1981 and Morton, 1981)
2.	Nakai	<i>Acanthopanax chiisanensis</i> Araliacae	Extract of root	(Lee et al.,2004)
3.	Gunja	<i>Abrus precatorious</i> Fabaceae	Leaves	(Toneli et al.,1965)
4.	Bilva	<i>Aegle marmelos</i> Rutaceae	Fruits	(Rao et al.,2006)
5.	Mata-raton	<i>Albizia lebbeck</i> Mimosaceae	Bark	(Saha et al.,1966)
6.	Lahasun	<i>Allium sativum</i> Liliaceae	bulb of garlic	(Nadkarni and Nadkarni 1976)
7.	Kaju	<i>Anacardium occidentale</i> Anacardiaceae	Dried juice of the leaves	(Huang, 1999)
8.	Pineapple	<i>Ananas comosus</i> Bromeliaceae	Fruits	(Brien et al., 2004)
9.	Satavari	<i>Asparagus racemosus</i> Liliaceae	Aerial parts	(Patwardhan et al.,2005)
10.	Neem	<i>Azadirachta Indica</i> Meliaceae	Leaf, seeds and bark	(Kaushik et al.,2002)
11.	Mandarai	<i>Bauhinia variegata</i> Caesalpiniaceae	Buds, Leaves, Stem, Bark	(SheshadriShekar et al.,2009)
12.	Huang Lian	<i>Berberis vulgaris</i> Berberidaceae	Stem, root bark, and fruit	(Chopra et al.,2004)

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S. No.	Common name	Botanical Name	Parts Used/ Extract	References
13.	Kaempferia pandurata	<i>Boesenburgia pandurata</i> Zingiberaceae	Oil	(Tuchinda <i>et al.</i> ,1965)
14.	Indian blibanum	<i>Boswellia serrata</i> Burseraceae	gum oleoresin	(Kimmatkar <i>et al.</i> ,2003)
15.	Kamani	<i>Calophyllum inophyllum</i> Clusiaceae	Oil	(Shen, 1981)
16.	Chinese tea	<i>Camellia sinensis</i> Theaceae	Used as tea	(Adcocks <i>et al.</i> ,2002)
17.	Golden shower	<i>Cassia fistula</i> Caesalpiniaceae	Whole Plant, Bark	(Anonymous, 1997)
18.	Tayuya, Tomba	<i>Cayaponia tayuya</i> Cucurbitaceae	Roots	(Escandell <i>et al.</i> ,2007)
19.	Indian pennywort	<i>Centella asiatica</i> Apiaceae	Leaves and aerial parts.	(Liu <i>et al.</i> ,2008)
20.	Amarello	<i>Chlorophora tinctoria</i> Anacardiaceae	seeds, nuts,	(Rotelli <i>et al.</i> ,2003)
21.	Guggul	<i>Commiphora mukul</i> Burseraceae	Gum	(Sharma <i>et al.</i> ,1977)
22.	Camphor	<i>Cinnamomum camphora</i> Lauraceae	Oil	(Lee <i>et al.</i> ,2006)
23.	Job's tears	<i>Coix lachrymal</i> Poaceae	Roots	(Otsuka <i>et al.</i> ,1988)
24.	Saffron	<i>Crocus sativus</i> Iridiaceae	Flower	(Hosseinzadeh and Younessi 2006)
25.	Dragons Blood	<i>Croton lechleri</i> Euphorbiaceae	Flower	(Perdue <i>et al.</i> ,1979)
26.	Sugi	<i>Cryptomeria japonica</i> Taxodiaceae	Wood oil	(Scudiero <i>et al.</i> ,1988)
27.	Gulmohar	<i>Caesalpinia ulcherrima</i> Leguminosae	Leave, Roots, Flower	(Patel <i>et al.</i> ,2010)
28.	Haldi	<i>Curcuma longa</i> Zingiberaceae	Rhizomes	(Aggarwal <i>et al.</i> ,2006)
29.	Shalaparni	<i>Desmodium gangeticum</i> Fabaceae	Aerial parts	(Mehrotra <i>et al.</i> ,2006)
30.	Lord William.	<i>Dianthus barbatus</i> Caryophyllaceae	Leaves	(Anna, 1984)
31.	Wild Yam	<i>Dioscorea deltoide</i> Dioscoreaceae	Roots	(Chatterjee and Das 1996)
32.	Wood Fern	<i>Dryopteris crassirhizoma</i> Dryopteridaceae	Rhizomes	(Chang <i>et al.</i> ,2010)
33.	Sehunda	<i>Euphorbia nerifolia</i> Euphorbiaceae	Leaves	(Alcaraz <i>et al.</i> ,1988)
34.	Cucumber	<i>Ecballium elaterium</i> Cucurbitaceae	Roots	(Tagarelli <i>et al.</i> ,2010)

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S. No.	Common name	Botanical Name	Parts Used/ Extract	References
35.	Gutta, Gamba.	<i>Garcinia cambogia</i> Clusiaceae	Gum resin.	(Mahendran <i>et al.</i> , 2002)
36.	Soybean	<i>Glycine max</i> Fabaceae	Seeds	(Verdrengh <i>et al.</i> , 2003)
37.	Mulathi	<i>Glycyrrhiza glabra</i> Leguminoseae	Rhizomes	(Tokiwa <i>et al.</i> , 2004)
38.	Nagadanti	<i>Heliotropium indicum</i> Boraginaceae	Root, Leaves	(Sriniwas <i>et al.</i> , 2000)
39.	Bola	<i>Hibiscus tiliaceus</i> Malvecae	Leaves	(Raghunathanand Sulochana 1994)
40.	Kokilaksha	<i>Hygrophila spinosa</i> Acanthaceae	Flower, Roots	(Chopra <i>et al.</i> , 1986; Nadkarni, 1978)
41.	Shyamla	<i>Ichnocarpus frutescens</i> Apocynaceae	Leaves, Stems	(Anonymous, 1956; Kirtikar and Basu 1999)
42.	Behaya	<i>Ipomoea fistulosa</i> Convolvulaceae	Leaves, Seeds	(Turner and Hebban 1965)
43.	Flaxseed	<i>Linum usitatissimum</i> Linaceae	SeedsJuice	(Nordstrom <i>et al.</i> , 1995)
44.	Chaturangi	<i>Lantana camara</i> Verbenaceae	Leaves, Aerial part, Flower	(Garg <i>et al.</i> , 1997)
45.	Ben teak	<i>Lagerstroema lanceolata</i> Lythraceae	Seeds	(Nadkarni, 1997)
46.	Butter tree	<i>Madhuca longifolia</i> Sapotaceae	Rhizomes	(Huang, 1999)
47.	Tulip	<i>Magnolia salicifolia</i> Magnoliaceae	Leaves	(Kimura <i>et al.</i> , 1985)
48.	Clock Plant	<i>Malvestrum coromandelianum</i> Malvaceae	Aerial part	(Rosa, 1972)
49.	Bitter melon	<i>Momordica charantia</i> Cucurbitaceae	Fruit	(Grover and Yadav 2004)
50.	Canary wood Kura	<i>Morinda citrifolia</i> Rubiaceae	Leaves	(Cheryl, 2007)
51.	Nutmeg	<i>Myristica fragrans</i> Myristaceae	Fruit	(Wang <i>et al.</i> , 1999)
52.	Tulsi	<i>Ocimum sanctum</i> Lamiaceae	Roots	(Nadkarni and Nadkarni 1976)
53.	Ginseng	<i>Panax ginseng</i> Araliaceae	Roots	(Kin <i>et al.</i> , 2010)
54.	Ren Shen	<i>Panax japonica</i> Araliaceae	Roots	(Dharmananda, 2002)
55.	Jangli amlı	<i>Phyllanthus amarus</i> Euphorbiaceae	Whole plant	(Mahat and Patil 2007)

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S. No.	Common name	Botanical Name	Parts Used/ Extract	References
56.	Myrobalan	<i>Phyllanthus emblica</i> Rubiaceae	Fruit, Leaves	(Sumanantranet al.,2007)
57.	Indian Comphor weed	<i>Pluchea indica</i> Asteraceae	Whole plant	(Sen and Chaudhari 1991)
58.	Rasna	<i>Pluchea lanceolata</i> Asteraceae	Leaves	(Chawla et al.,1992)
59.	White-flower leadwort	<i>Plumbago zeylanica</i> Plumbaginaceae	Leaf, roots	(Oyedapo, 1976)
60.	Castor	<i>Ricinus communis</i> Euphorbiaceae	leaves	(Parkkinen, 1989)
61.	Romero	<i>Rosmarinus officinalis</i> Lamiaceae	Essential oil from the aerial parts	(Youn, 2003)
62.	Burans	<i>Rhododendron arboreum</i> Ericaceae	leaves	(Sharma et al.,2009)
63.	Baical skullcap	<i>Scutellaria baicalensis</i> Lamiaceae	Aerial parts	(Butenko et al.,2003)
64.	Marking nut	<i>Semecarpus anacardium</i> Anacardiaceae	Fruits , Gum	(Vijaylakshmi et al.,1997; Satyavathi et al.,1969)
65.	Egyptian sesban	<i>Sesbania sesban</i> Fabaceae	Leaves	(Wang et al.,2008)
66.	Bala	<i>Sida cordifolia</i> Malvaceae	Leaves	(Franzotti et al.,2000)
67.	Thistle	<i>Silybum marianum</i> Asteraceae	Whole herb	(Gupta et al.,2000)
68.	Paccotti	<i>Simplocos spicata</i> Symplococeae	Extract of leaves	(Froton et al.,1983)
69.	Mawaengkrue	<i>Solanum trilobatum</i> Solanaceae	Roots	(Panduragan et al.,2011)
70.	Han fang ji	<i>Stephania tetrandra</i> Menispermaceae	Whole plant	(Kang et al.,1996)
71.	Marigold	<i>Tagetes erectus</i> Asteraceae	Leaves	(Kirtikar and Basu 1993)
72.	Fever Few	<i>Tanacetum parthenium</i> Asteraceae	Leaves	(Pattrick et al.,1989)
73.	Harad	<i>Terminalia chebula</i> Combretaceae	Fruits	(Nair et al.,2010)
74.	Black afara	<i>Terminalia ivorensis</i> Combretaceae	Leaf	(Iwu and Anyanwu 1982)
75.	Echte gamamder	<i>Teucrium chamaedrys</i> Lamiaceae	Leaves	(Mary and Keifer 1998)
76.	Garden Thyme	<i>Thymus vulgaris</i> Lamiaceae	Leaves	(Kumazawa et al.,2006)

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for Anti-inflammatory and Anti-arthritis activity

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S. No.	Common name	Botanical Name	Parts Used/ Extract	References
77.	Gokhru	<i>Tribulus terrestris</i> Zygophyllaceae	Whole Plant, Seeds	(Raza, 1975)
78.	Ipecac	<i>Tylophora indica</i> Asclepiadaceae	Roots	(Shah <i>et al.</i> , 2006; Shen, 1981)
79.	Emetic Swallow-wory	<i>Tylophora asthmatica</i> Asclepiadaceae	Roots	(Thiruvengadam <i>et al.</i> , 1981)
80.	Ashwgandha	<i>Withania somnifera</i> Solanaceae	Leaves	(Begum and Sadique, 1988; Sumantran <i>et al.</i> , 2007)

1.6.2 Phytoconstituents reported to have anti-inflammatory activity:

Natural chemical agents extracted from plants that can modulate the expression of pro-inflammatory signals clearly have potential against arthritis. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins, polyphenols and anthoxanthins, all of which are known to have anti-inflammatory effects.

The phytoconstituents which have been attributed with anti-inflammatory activity are:

1. Alkaloids: Thalicsiline (Diterpenoid alkaloid), Cycleanine and Tetranidrine, Rohutkin alkaloid, Trilobine and Isotrilobine etc (Ferrante *et al.*., 1990; Tehet *et al.*., 1990).
2. Triterpenoids and their glycosides: Aescin (β -amyrin), Chisanosides (lupine triterpenoids), Dysobinin, Boswellic acid and pentacyclic triterpenoid acids, α -amyrin & Taxifolin 3, Sorghumol, Bassic acid etc (Juteaua *et al.*, 2002).
3. Flavonoids & Coumarins: Hypoleitin & Sideritoflavone, Baicalin, Baicalein, 5, 7-dimethoxyflavone, Osthol (Coumarin), Quercetin-3-o-rhamnoglucoside, Kaempferol, Hedychinone (Flavonoid), Marmin (Coumarin) etc (Tubaro *et al.*, 1989).
4. Saponin and saponins: Phytolaccoside B (saponin), Panax Saponin, Micosaponins , Saikogenin, Glycyrrhytinic and Glycyrrhizinic acids etc (Lanherset *et al.*., 1992).
5. Steroidal components: Spinasterol, B -Sitosterol, Steroidal components of Boughainwellia glabra etc (Yunes *et al.*, 1992).
6. Xanthones & their glycosides: Calophyllolide, Magniferin, a Xanthone-C-glycoside, Xanthorhamnin etc (Lanherset *et al.*., 1992).

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7. Others: Magnoshinin, Hematoxylin, Copalifera Oleoresin, pinens, Benzoxacinoid compounds, Bavachinin, Gangetin, Embelin, Epicatechin etc (Ammon *et al.*.,, 1991).

1.7 OBJECTIVES OF THE STUDY

The aim of the study was to identification, authentication and review of literature of medicinal plants. To conduct pharmacognostic evaluation of *Ficus lacor* aerial roots and *Murraya koenigii* roots. To carry out phytochemical investigations on *Ficus lacor* aerial roots and *Murraya koenigii* roots. To carry out polarity based extraction using different solvents. Estimation of anti-inflammatory and anti-arthritis potential of isolated extracts/ fractions using different animal models. To estimate articular damage and its inhibition by extracts and in vitro anti-oxidant activity of plants extract/ fractions.

1.8 Review of Literature *Murraya koenigii*

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. (Anonymous, 1997). *Murraya koenigii* Linn., commonly known 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. The traditional system of medicine, it is utilized as blood purifier, anti-diarrhoeal, dysentery, tonic, stomachic, febrifuge, antiemetic, in curries and chutneys as a flavouring agent (Anonymous, 1998; Prajapati *et al.*, 2003).

1.8.1 Taxonomic Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: Murraya

Species: koenigii



Figure 1.5: *Murraya koenigii* Plant

1.8.2 Synonyms: Hindi: Mitha neem, Curry patta, Sanskrit: Krishna nimbi, Assamese: Narsinghs, Bisharhari, Bengali: Barsanga, Kariphulli, Punjabi: Curry patta; Marathi: Poospala, Karhinimb, Gandla, ,Gujarati: Kadhilimbdo, Goranimb; Kannada: Karibevu, Malayalam: Karriveppilei, Oriya: Basango, Barsan, Bhuraunga; Tamil: Karivempu, Karuveppilei, Telugu: Karepaku (Iyer and Devi 2008).

1.8.3 Morphological characters

It is a small spreading shrub, approx 2.5 metres height, with the green to brownish, with several dots on it, bark can be unpeel longitudinally, exposing the white wood underneath. Stems have a characteristic aromatic sweet odor. The surface is smooth, soft and glabrous (Pandey *et al.*, 2009). The mature unpeeled stems are dark brown in colour. The peeled stem creamish brown in colour. The odour aromatic with characteristic taste. The outer surface is smooth but hard with splintery fracture (Jerald *et al.*, 2008).

Leaves: Leaves are in green colour and typical odour. Leaves are exstipulate, bipinnately compound, thirty cm long, each bearing twenty four leaflets, having

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reticulate venation; leaflets, lanceolate, 4.9 cm length, 1.8 cm broad, having 0.5-cm-long petiole (Roy, 1995).

Flowers: The flowers are bisexual, white, funnel-shaped, sweet aromatic, stalked, consummate, ebracteate, conventional (regular), hypogynous, pentamerous, actinomorphic, the normal diameter of a planarity opened flower 1.12 cm; inflorescence (terminal cyme) each demeanor 60 to 90 flowers bunch, calyx, 5-lobed, sedulously assiduous, inferior, green, corolla, white, many petalous, inferior, with 5 petals, lanceolate, length, 5 mm, androecium, inferior, polyandrous, with 10 stamens, dorsifixed, prearranged into circular manner of five each, more minute stamens, 4 mm length , the gynoecium, 5 to 6 mm long, stigma, effulgent, sticky, style, short ovary, superior (Khosa and Prasad 1972).

Fruits: The fruits are round to oblong shape, 1.4 to 1.6 cm long, 1 to 1.2 cm in thickness; plenarily ripe fruits, ebony with a very shining surface. One seed in each fruit, 11 mm long, 8 mm in thick, color spinach green. December to July Flowers and fruiting occurs. This suckering plant can grow to a tree up to 6 metres height in warm, sultry climates, but it can additionally be grown very prosperously in a pot as a much more diminutive plant (Roy, 1995; Khosa and Prasad 1974).

It is generally more minuscule if grown out of its mundane climate zone. The acerbically - flavoured pinnate leaves are borne on antithesis tenuous branch lets and have an unorthodox adornment habit. The leaves are smooth silky and glossy with paler undersides. (Khosa *et al.*, 1970).

1.8.4 REPORTED PHARMACOLOGICAL STUDIES

1.8.4.1 Antibacterial activity

Three bioactive carbazole alkaloids named as, mahanine murrayanol and mahanimbine, isolated from leaves, which has shown antimicrobial, mosquitocidal, and topoisomerase I & II inhibition activities (Narasimhan *et al.*, . 1975). The essential oil obtained from leaves show anti-bacterial against *Bacillus subtilis*, *Staphlococcus aureus*, *Corynebacterium pyogenes*, *Proteus vulgaris* and *Pasteurella multicida*. The oil was active against the first mentioned three micro-organisms. The minimum inhibitory concentrations of alkaloidal and steroid compounds of stem bark were found to be in the range 3.13-100 microg/ml (Rahman and Gray 2005).

The essential oil has been isolated from leaves of *Listeria innocua*. The constituents reported are α -copaene, β -gurjunene, iso-caryophyllene (12.1%), beta-caryophyllene

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(8.7%) and germacrene D. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of both isolated essential oils were low about 10-25%. The essential oil at 300 microg/mL provided 92% inhibition (Erkan *et al.*, 2012).

1.8.4.2 Antifungal potential

The essential oil isolated from *Murraya koenigii* leaves showing antifungal potential against *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Aspergillus fumigatus* and *Microsporum gypseum*. It was found that effective against *Candida albicans* at a dilution of 1:500. The ethanol extract of the leaves shown fungotoxicity against *Rhizoctonia solani* and *Colletotrichum falcatum* (Kishore *et al.*, 1982). *M. Koenigii* aqueous and ethanol extracts of leaves were evaluated for the anti-candidal activity against the *Candida albicans* (Vaijayanthimala *et al.*, 2000).

1.8.4.3 Larvicidal activity

Acetone and Petroleum ether extracts isolated from *Murraya koenigii* leaves, at a concentration of 250-900 ppm showed Larvicidal activity against *A.aegypti* larvae. (Harve *et al.*, 2004).

1.8.4.4 Anti-diabetic and Hypoglycaemic activity

The defensive impact of *M. koenigii* leaves separate against α -cell harmed and hostile to oxidant barrier arrangement of plasma and pancreas in streptozotocin actuated diabetic rats was demonstrated a defensive impact in diabetes by declining oxidative anxiety and pancreatic α -cell devastation (Arulsevacn and Subramanian 2007). The water concentrate of the *M. koenigii* leaves demonstrated the blood glucose level diminishing in diabetic rats and alloxan prompted diabetic rabbits with the impact of a standard tolbutamide hypoglycaemic medication. The anti-diabetogenic impacts of *Murraya koenigii* (L.) Spr. furthermore, *Ocimum tenuiflorum* L. on streptozotocin-prompted diabetic Swiss mice. Treatment with chloroform concentrates of *Murraya koenigii* (MKC) and watery concentrate of *Ocimum tenuiflorum* (OTA) brought about legitimate glucose usage with an intensify in liver glucose-6-phosphate dehydrogenase compound action and glycogenesis in ordinary liver and muscle tissues. Pancreatic and intestinal glucosidase inhibitory movement saw with MKC and OTA treatment showed useful impacts in decreasing postprandial hyperglycemia with accompanying change in glucose digestion system. The histochemical and immunohistochemical investigation of pancreatic islets recommends the part of MKC and OTA in pancreatic β -cell assurance and the practical pancreatic islets that deliver

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insulin. The study shows the criticalness of MKC and OTA in glucosidase restraint and islet insurance in the murine diabetic model. These discoveries recommend the capability of the concentrates in adjuvant treatment for the treatment of diabetes and the conceivable improvement of potential neutraceutic (Dusane and Joshi 2012).

1.8.4.5 Antiprotozoal activity

Ethanol extracts of aerial parts of *Murraya koenigii* plant, excluding roots and roots alone were screened for their pharmacological actions. Whole plant extract showing antiprotozoal activity against *Entameoba histolytica*, anti-spasmodic effect on isolated ileum of guinea pig, the roots extract showed antiprotozoal activity against *Ent. Histolytica*, as well as antihypertensive activity in dogs (Bhakuni *et al.*, 1969).

1.8.4.6 Anti-inflammatory and Analgesic activity

The ethanol extract of leaves of *Murraya koenigii* showed anti-histaminic and anti-inflammatory effects (Parmar *et al.*, 2010; Darvekar and Patil 2011). The methanol extract shown significant reduction in carrageenan induce paw edema and analgesic activities (Gupta *et al.*, 2010).

1.8.4.7 Antioxidant activity

The antioxidant properties of the extract of *Murraya koenigii* leaves were screened on the basis of stability index of oil and free radical scavenging ability against DPPH. Methylene chloride extract and Ethyl acetate fraction of the seventy percent acetone extract was extended the OSI values significantly as compared to α -tocopherol and BHT. The five carbazole alkaloids have been isolated from the methylene chloride extract and identified as Bismurrayafoline, Euchrestine, Mahanine, and Mahanimbine based on MS and NMR spectra (Tachibana *et al.*, 2001) and the extract also showed scavenging of NO and exhibited significant activity (Baliga *et al.*, 2003).

1.8.4.8 Haematological studies

The *Murraya koenigii* leaves different extracts were screened for haematological parameters. They do not show any side effects on food efficiency ratio, white blood cells, red blood cell count , total count and blood components like serum haemoglobin, electrolytes, blood urea, fibrin level, total serum protein, glycosylated haemoglobin and the activity of glutamic pyruvic transaminase, glutamic oxaloacetic transaminase and alkaline phosph in serum (Khan *et al.*, 1995).

1.8.4.9 Hypcholestremic potential

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Biochemical replication in wistar rats was screened by supplementation of curry leaves to the diet. Albino rats were alimented for 90 days on a typical lab rat diet plus 20 percent coconut oil either alone or with the additament of 10 percent curries leaf. Aliment was offered at a caliber of 10 percent body weight. The leaf shwon in the reduction in total serum cholesterol, LDL, VLDL an incrementation in the HDL, lower relinquishment of lipoproteins into the circulation and an increase in the LCAT activity (Khan *et al.*, 1996).

Murraya koenigii and *Brassica juncea* seeds were studied for effects on the lipids contents, sterol and neutral fecal bile acids in rats given with DPPH and showed decreases in the calibers of cholesterol and phospholipids in the experimenter when compared with the control group (Khan *et al.*, 1995).

1.8.4.10 Anti-Hypertensive activity

The angiotensin converting enzyme inhibitor and the antihypertensive activity of natural products containing a crust of a seed of *Hymenaea courbaril*, a leaves of guava, *Murraya koenigii*, *Tomarix chinensis* Lour, a leaves of *Morus bombycina*, extract of *Mimusops elengi* and formulation of the conshiolin with succinic anhydride have reported (Takagi and Shimomura 2004). An ethanol extract of fresh leaves of *Murraya koenigii* shwon dose dependent positive inotropic acivity on the isolated frog heart by escalating availability of calcium from extracellular sites (Shah and Juvekar 2006).

1.8.4.11 Cytotoxic activity

Carbazole alkaloids Mahenine, Pyrayafoline-D and Murrafoline-1 showed important cytotoxicity against HL-60 cells. The fluorescence microscopy by means of Hoechst 33342 staining discovered that the percentage of apoptotic cells with fragmented nuclei and condensed chromatin have been increased in a time dependent approach after treatment with every alkaloid (Ito *et al.*, 2006).

1.8.4.12 Anti-diarrhoeal

Three bioactive carbazole alkaloids were isolated from the n - hexane extract of seeds of *Murraya koenigii* spring (kurryam (I), koenimbine (II) and koenine (III)). Out of the three compounds, (I) and (II) showed significant inhibitory activity in opposition to castor oil-induced diarrhoea and PGE-2 induced enteropooling in rats (Mandal *et al.*, 2010).

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1.8.4.13 Hepatprotective potential

The hepatoprotective capability of polyphenol rich *Murraya koenigii* L. (MK). The hydro-ethanolic leaf separate in CCl₄ was for treating hepatotoxic rats. Plasma markers of hepatic harm, lipid peroxidation levels, enzymatic, and non-enzymatic cell reinforcements in liver and histopathological changes were explored in control and treated rats. MK pretreated rats with distinctive measurements (i.e 200, 400 and 600mg/kg bw) demonstrated huge diminishing in movement levels of alanine aminotransferase, aspartate aminotransferase, antacid phosphatase, all out protein, and bilirubin. MK treated rats recorded a measurement subordinate addition in hepatic super oxide dismutase, catalase, lessened glutathione and ascorbic corrosive and, a decrement in lipid peroxidation. Minuscule assessments of liver uncovered CCl₄ affected sores and related harmful indications that were insignificant in liver of rats pretreated with *Murraya koenigii* extricate (Desai *et al.*,2012) and at the dosage of 100 µg/ml and 500 µg/ml of fluid leaves concentrate, detached carbazole alkaloids (CA) and tannin part additionally demonstrated liver ensuring impact against ethanol-affected hepatotoxicity through liver carcinoma cell lines. The cell reinforcement movement with against lipid peroxidation potential, consequences for protein substance and, liver metabolizing chemicals the histology of the cells had been concentrated on as parameters of hepatoprotection (Sathaye *et al.*,2011).

1.8.4.14 Trypsin inhibitor

Murraya koenigii extract showed trypsin inhibitor activity. Trypsin revealed a compact structure composed of central beta sheet circumvented by α -helices with variation in structural practical stability and *Murraya koenigii* extract showed correlating reduction in inhibitory potential and helical components at incrementing temperature suggesting a possible role for α -helical structure in inhibitory function of the protein (Shree *et al.*,2006).

1.8.4.15 Immunomodulatory activity

The methanol extract of *Murraya koenigii* leaves was screened in cell mediated and humoral immune response to ovalbumin, phagocytic action by carbon consent test, nitric oxide liberate from murine peritoneal macrophages, cyclophosphamide induced myelosuppression. A significant increase in the Nitric oxide production by rats peritoneal macrophages was seen in culture supernatants representing the increased phagocytic activity of macrophages. The extract have been showed significant

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amplify in phagocytic index by rapid exclusion of carbon particles from blood flow. The extract furthermore increased the antibody titer against ovalbumin and safety towards the cyclophosphamide induce myeloma suppression (Shah *et al.*,2008).

1.8.4.16 Pancreatic lipase inhibitory activity

A total sixty three extracts from different 21plants were screened to evaluation of in vitro pancreatic lipase inhibitory activity. But all three extracts of *Murraya koenigii* (L.) Spreng leaves exhibited anti-lipase activity more than 80 percent. the isolation of four alkaloids from extract fractination, viz. Koenimbin, Mahanimbin, Clausazoline-K and Koenigicine. It is calculated with IC₅₀ values of 17.9 mM, 168.6 mM, 428.6 mM and <500 mM, respectively (Birari *et al.*,2009).

1.8.4.17 Nephroprotective activity

Daily oral administration of various dose levels of the aqueous extract for 30 days, produced decrease in serum urea and creatinine levels significantly, and significant increment in the levels of serum antioxidant potency in diabetic treated rats, compared to the control groups. These animals shown equivalent tissue revival by the extract in histological studies of the kidneys (Wang *et al.*,2003).

1.8.4.18 Anti-osteoporotic

New carbazole alkaloids i.e. murrayanine and 8, 8-biskoenigine were extracted from *Murraya koenigii*. The compound 8, 8-biskoenigine was a symmetrical dimer of the koenigine and shown anti-osteoporotic potential in the CAT-B model with IC₅₀ 1.3 µg/ml (Wang *et al.*,2003).

1.8.4.19 Wound healing

The aqueous extract of *Murraya koenigii* leaves showed significant reduction in wound area in comparison with control group from fourth day onwards in rats by excision wound model (Patidar *et al.*,2010).

1.8.4.20 Anti-amnesic activity

The diets of *Murraya koenigii* leaves engendered a paramount dose-dependent amendment in the memory scores of aged mice. The significant reduction in the amnesia induced by diazepam and scopolamine. Additionally, brain cholinesterase action and total cholesterol quantities were decreased by the *Murraya koenigii* leaves diets. (Vasudevan and Parle 2009) and also the effect of total alkaloidal extract was found to be significant at the dose of 20 and 30 mg/kg, p.o., improvement in memory scores of young and aged mice (Mani *et al.*,2012).

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1.8.4.21 Anti-tumour potential

A carbazole alkaloid Girinimbine was isolated from the bark of stems of *Murraya koenigii* and tested for in vitro anticancer and antioxidant activities. Anti-tumour activity was tenacious by assaying the capability of the compound to inhibit the term of early on antigen of raji cells (Epstein-Barr virus) that was induced by the tumour promoter (12-O-tetradecanoylphorbol-13-acetate). Girinimbine produced significant anti-oxidant activity as compared with standard a-tocopherol (Kok *et al.*, 2012).

1.8.4.22 Acetylcholinesterase inhibitory activity:

Mahanimbine was isolated from the leaves of *Murraya koenigii*. Mahanimbine had AChE inhibition activity in a dose-dependent method (Kumar *et al.*, 2010).

1.8.5 Reported Phytoconstituents

Table 1.2: List of Reported Phytoconstituents of *Murraya koenigii*

S. No.	Chemical constituents	Parts/ Extracts	References
1.	Koenimbine	Seeds, leaves and stem bark	(Joshi <i>et al.</i> , 1970; Narasimhan <i>et al.</i> , 1975; Adesina <i>et al.</i> , 1994)
2.	Koenoline	Roots bark	(Fiebig <i>et al.</i> , 1985)
3.	Murrayacine	Stem bark	(Sukari, 2001; Kureel <i>et al.</i> , 1970)
4.	Girinimbine	Stem bark, roots and seeds	(Rao <i>et al.</i> , 1980; Reisch <i>et al.</i> , 1994)
5.	Koenimidine	Roots and leaves	(Joshi <i>et al.</i> , 1970; Narasimhan <i>et al.</i> , 1975; Narasimhan, 1970)
6.	Koenine	Leaves	(Narasimhan <i>et al.</i> , 1975; Saha & Chaudhary, 1998)
7.	Koenigine	Leaves	(Narasimhan <i>et al.</i> , 1975, Wang & He, 2002)
8.	Mukonicine	Leaves	(Mukharjee <i>et al.</i> , 1983)
9.	Mahanimbine	Seeds, leaves and roots	(Ramsewak <i>et al.</i> , 1999; Tachibana <i>et al.</i> , 2001)
10.	Mahanine	Seeds and leaves	(Ramsewak <i>et al.</i> , 1999; Tachibana <i>et al.</i> , 2001)

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S. No.	Chemical constituents	Parts/ Extracts	References
11.	Mahanimbinine	Seeds	(Nutan <i>et al.</i> ,1999)
12.	Murrayacine	Leaves	(Reisch <i>et al.</i> ,1994)
13.	Mahanimbicine	Roots and Leaves	(Tachibana <i>et al.</i> ,2001)
14.	Mahanimboline	Roots	(Roy <i>et al.</i> ,1979)
15.	Isomahananine	Seeds	(Adesina <i>et al.</i> ,1994)
16.	Kurryam	Seeds	(Mandal <i>et al.</i> ,2008)
17.	Curryangin or Murrayazoline	Leaves	(Dutta <i>et al.</i> ,1969; Bhattacharya <i>et al.</i> ,1989)
18.	Murrayazolinol	Stem bark and roots	(Bhattacharya <i>et al.</i> ,1989)
19.	Isomurrayazoline	Stem bark	(Bhattacharya <i>et al.</i> ,1982)
20.	Cyclomahanimbine	Stem bark and leaves	(Kureel <i>et al.</i> , 1969)
21.	Murrayazolinine	Stem bark	(Bhattacharya <i>et al.</i> ,1982)
22.	Isomurrayazolinine	Stem bark	(Bhattacharya <i>et al.</i> ,1982)
23.	Bicyclomahanimbine	Leaves	(Kureel <i>et al.</i> ,1970; (Rao <i>et al.</i> ,1980; Kureel <i>et al.</i> , 1969)
24.	Bicyclomahanimbicine	Leaves	(Kureel <i>et al.</i> , 1970)
25.	Mukoeic acid	Stem bark	(Wang & He, 2003)
26.	Murrayanine	Stem bark	(Wang & He, 2003; Roy <i>et al.</i> , 1982)
27.	Mukonine	Stem bark	(Bhattacharya <i>et al.</i> ,1982)
28.	Mukonidine	Stem bark	(Kureel <i>et al.</i> ,1969)
29.	Mahanimbinol	Stem bark	(Reisch <i>et al.</i> ,1994)
30.	Mukoline	Roots	(Roy <i>et al.</i> ,1982)
31.	Mukolidine	Roots	(Reisch <i>et al.</i> ,1994)
32.	3,6,-dimethyl-1-isopentenyl carbazole	Roots	(Begum <i>et al.</i> ,2005)
33.	Murrayanol	Seeds and leaves	(Ramsewak <i>et al.</i> ,1999)

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S. No.	Chemical constituents	Parts/ Extracts	References
34.	Grinimbilol	Stem bark	(Reisch <i>et al.</i> , 1994)
35.	Koenoline	Roots	(Fiebig <i>et al.</i> , 1985)
36.	Glycozoline	Roots	(Adesina <i>et al.</i> , 1988)
37.	3-Methyl cabazole	Roots	(Chakarborty <i>et al.</i> , 1997)
38.	2-Hydroxy-3-methyl cabazole	Roots	(Bhattacharyya <i>et al.</i> , 1986)
39.	Euchrestine	Leaves	(Kureel <i>et al.</i> , 1969)
40.	Murrayazolidine	Stem bark	(Chakarborty <i>et al.</i> , 1970; Chakarborty <i>et al.</i> , 1974)
41.	Bicyclomahanimiline	Stem bark	(Bhattacharyya <i>et al.</i> , 1984)
42.	Koenigine-quinone-A	Stem bark	(Saha & Chaudhary, 1998)
43.	Koenigine-quinone-B	Stem bark	(Saha & Chaudhary, 1998)
44.	9-Carboxy-3-methyl cabazole	Stem bark	(Chokarborty <i>et al.</i> , 1994)
45.	9-Formyl-3-methyl carbazole	Stem bark	(Chokarborty <i>et al.</i> , 1994)
46.	Bismurrayfoline	Leaves	(Tachibana <i>et al.</i> , 2001)
47.	8,8"- Bikoenigine	Leaves	(Wang & He, 2003)
48.	Scaoplin	Stem bark	(Chakarborty <i>et al.</i> , 1974)
49.	3-(1,1-dimethylallyl) Xanthyletin	Stem bark	(Bhattacharya <i>et al.</i> , 1984)
50.	Sabinene	Leaves	(Pandey <i>et al.</i> , 2009; Mallavarapu <i>et al.</i> , 1999)
51.	Trans- β -caryophyllene	Leaves	(Pandey <i>et al.</i> , 2004)
52.	α -pinene	Leaves	(Mallavarapu <i>et al.</i> , 1999; Pandey <i>et al.</i> , 2004)
53.	β -cubebene	Leaves	(Wagner <i>et al.</i> , 1993)
54.	β -gurjunene	Leaves	(Wagner <i>et al.</i> , 1993)
55.	β -caryophyllene	Leaves	(Wong & Ghee 1996)
56.	Cis-caryophyllene	Leaves	(Choudhary, 2000)
57.	Dipentene	Leaves	(Choudhary, 2000)

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S. No.	Chemical constituents	Parts/ Extracts	References
58.	α -eudesmol	Leaves	(Choudhary, 2000)
59.	Iso-caryophyllene	Leaves	(Choudhary, 2000)
60.	SS-elemene	Leaves	(Choudhary, 2000)
61.	α -phellendrene	Leaves	(Wagner <i>et al.</i> ,1993)
62.	β - phellendrene	Leaves	(Wagner <i>et al.</i> ,1993)
63.	(E)- β -ocimene	Leaves	(Mallavarapu <i>et al.</i> ,1999)
64.	(Z)- β -ocimene	Leaves	(Mallavarapu <i>et al.</i> ,1999)
65.	β -pinene	Leaves	(Wong & Tei 1993)
66.	Myrecene	Leaves	(Mallavarapu <i>et al.</i> ,1999)
67.	$\hat{\mu}$ -terpinene	Leaves	(Mallavarapu <i>et al.</i> ,1999)
68.	α - terpinene	Leaves	(Wong & Tei 1993)
69.	Terpinen-4-ol	Leaves	(Mallavarapu <i>et al.</i> ,1999)
70.	Linalool	Leaves	(Wong & Tei 1993)
71.	Xanthotoxin	Seeds	(Adebjo & Reisch 2000)
72.	Iso-byakangelicol	Seeds	(Adebjo & Reisch 2000)
73.	Phellopterin	Seeds	(Adebjo & Reisch 2000)
74.	Gosferol	Seeds	(Adebjo & Reisch 2000)
75.	Neo- byakangelicol	Seeds	(Adebjo & Reisch 2000)
76.	Byakangelicol	Seeds	(Adebjo & Reisch 2000)
77.	Byakangelicin	Seeds	(Adebjo & Reisch 2000)
78.	Isogosferol	Seeds	(Adebjo & Reisch 2000)
79.	β -sitosterol	Leaves	(Adebjo & Reisch 2000)
80.	β - elemene	Leaves	(Wagner <i>et al.</i> ,1993)
81.	D-limolinene	Leaves	(Srivastava & Ray 2007)
82.	Camphene	Leaves	(Wagner <i>et al.</i> ,1993)
83.	α -selinene	Leaves	(Wagner <i>et al.</i> ,1993)
84.	δ -cadinene	Leaves	(Wagner <i>et al.</i> ,1993)
85.	β -bisabolene	Leaves	(Wagner <i>et al.</i> ,1993)
86.	Caryophyllene epoxide	Leaves	(Wagner <i>et al.</i> ,1993)
87.	α -copene	Leaves	(Wagner <i>et al.</i> ,1993)
88.	Humulene	Leaves	(Wagner <i>et al.</i> ,1993)

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S. No.	Chemical constituents	Parts/ Extracts	References
89.	Selin-11-en-4 α -ol	Leaves	(Wagner <i>et al.</i> , 1993)
90.	α -cubebene	Leaves	(Wagner <i>et al.</i> , 1993)
91.	β -thujene	Leaves	(Wagner <i>et al.</i> , 1993)
92.	$\hat{\cup}$ -cadinene	Leaves	(Wagner <i>et al.</i> , 1993)
93.	Trans-sebinene hydrate	Fruits	(Mallavarapu <i>et al.</i> , 2000)
94.	Cis-sebinene hydrate	Fruits	(Mallavarapu <i>et al.</i> , 2000)
95.	Nerol	Fruits	(Mallavarapu <i>et al.</i> , 2000)
96.	E-nerolidol	Fruits	(Mallavarapu <i>et al.</i> , 2000)
97.	Spathulenol	Leaves	(Mallavarapu <i>et al.</i> , 2000)
98.	α , ρ -dimethyl styrene	Leaves	(Mallavarapu <i>et al.</i> , 2000)
99.	Livandulol	Leaves	(Mallavarapu <i>et al.</i> , 2000)
100.	Geraniol	Leaves	(Mallavarapu <i>et al.</i> , 2000)
102.	Bornyl acetate	Leaves	(Mallavarapu <i>et al.</i> , 2000)
103.	Neryl acetate	Leaves	(Mallavarapu <i>et al.</i> , 2000)
104.	Cis- β -guaenine	Leaves	(Mallavarapu <i>et al.</i> , 2000)
105.	EE-fernesol	Leaves	(Mallavarapu <i>et al.</i> , 2000)
106.	T-cadinol	Leaves	(Mallavarapu <i>et al.</i> , 2000)
107.	Tricyclene	Leaves	(Mallavarapu <i>et al.</i> , 2000)
108.	Murolene	Leaves	(Wagner <i>et al.</i> , 1993)

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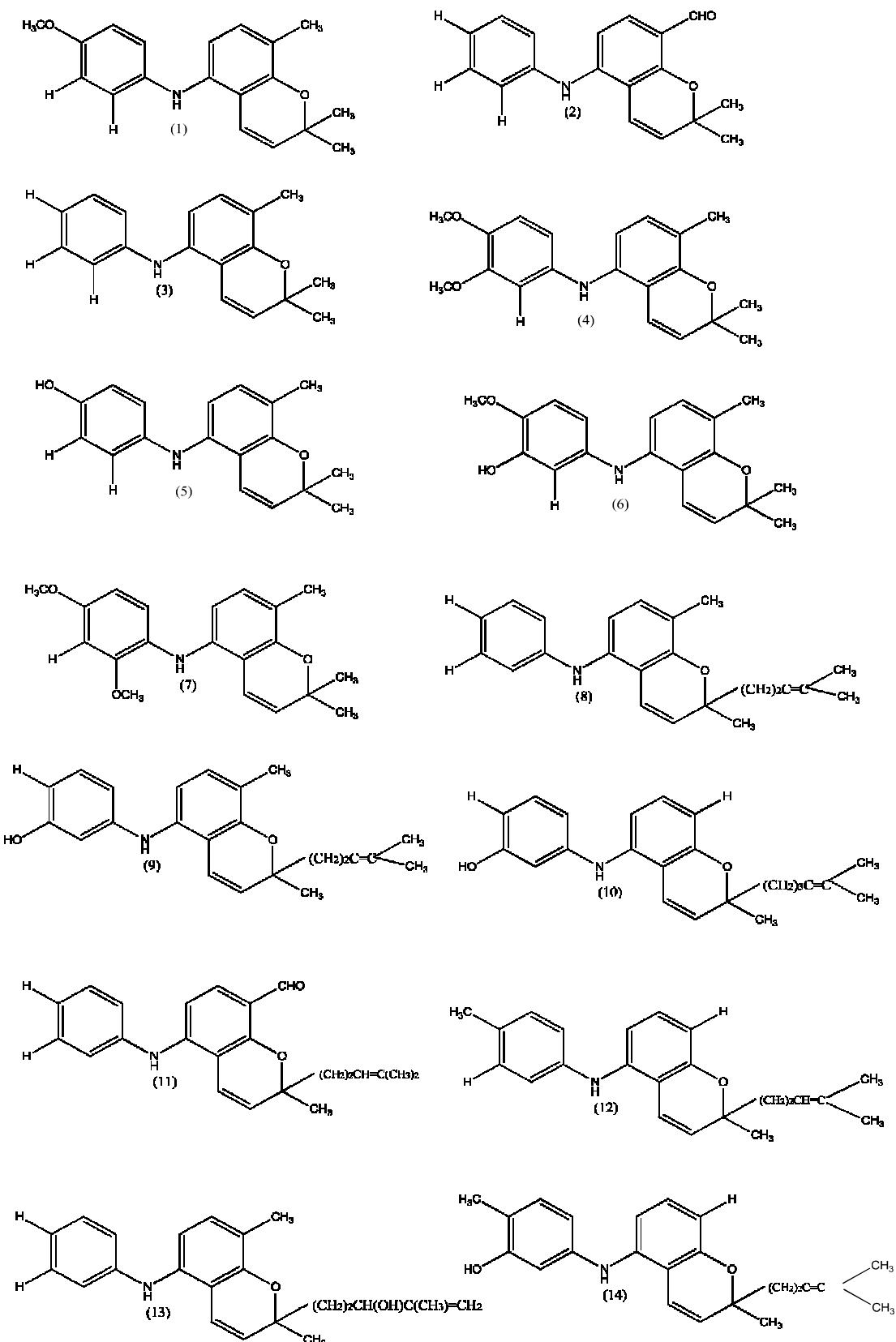


Figure 1.6 Reported phytoconstituents of *Murraya koenigii*

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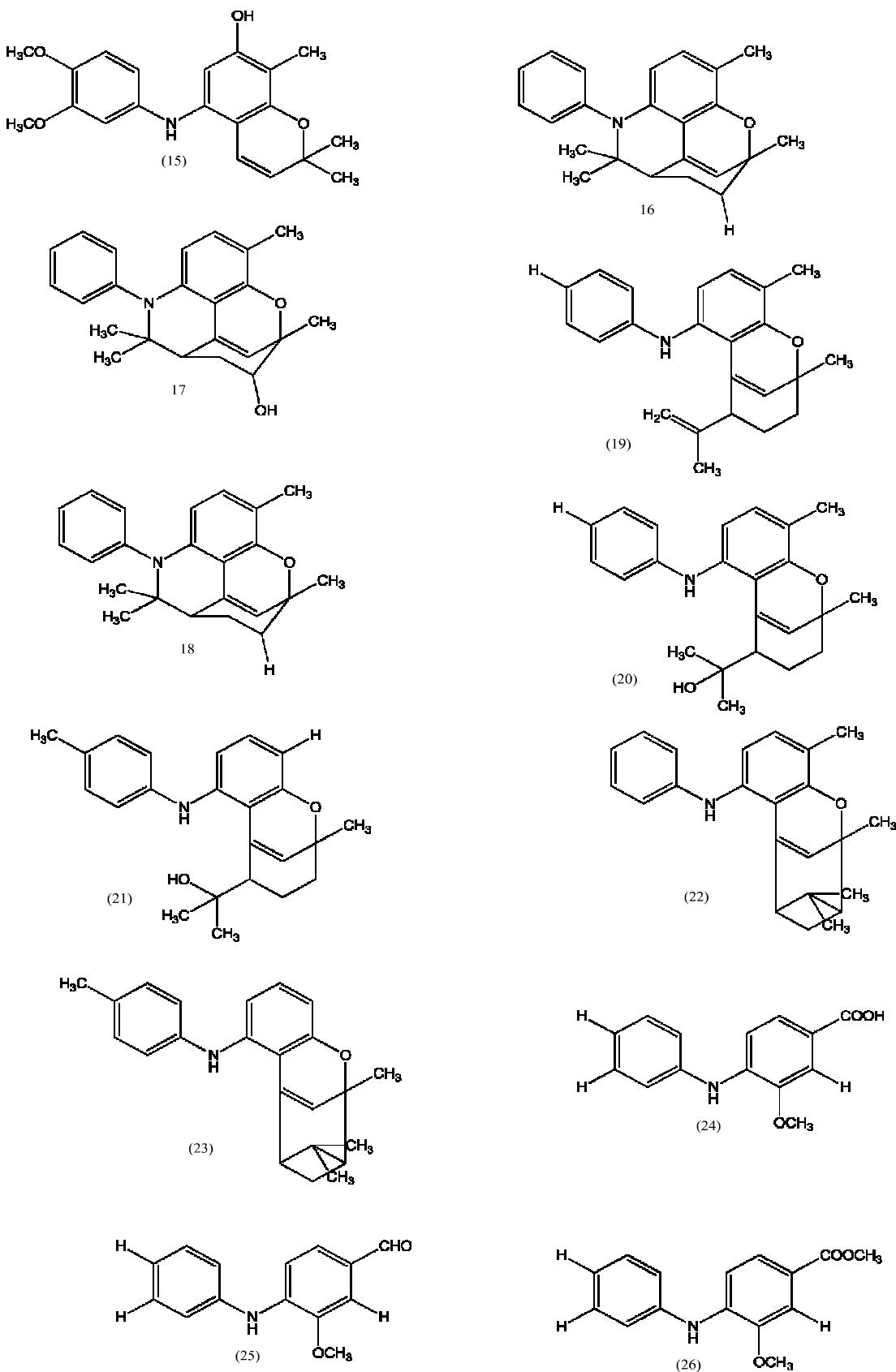


Figure 1.7: Reported phytoconstituents of *Murraya koenigii*

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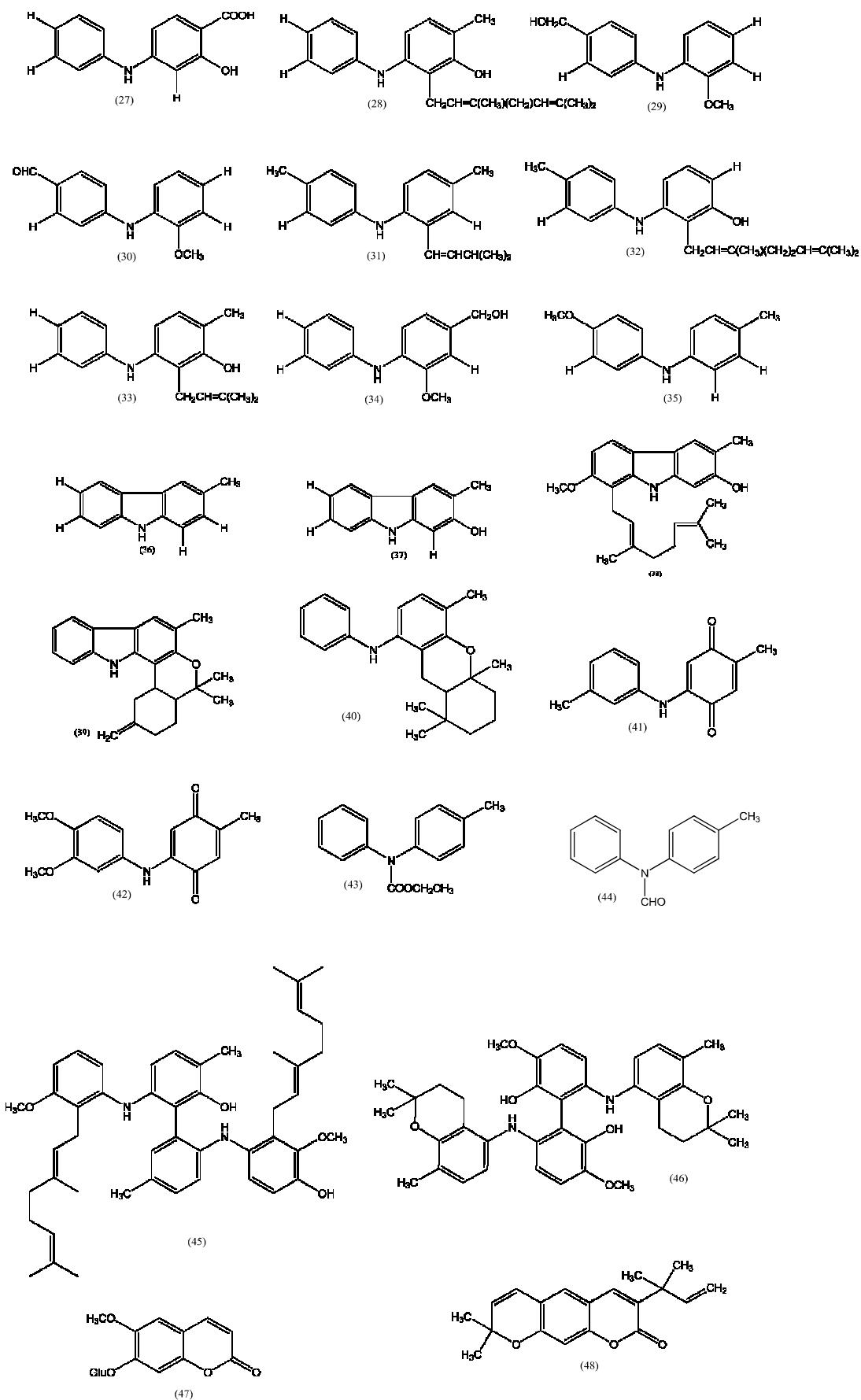


Figure 1.8: Reported phytoconstituents of *Murraya koenigii*

1.9 Review of literature *Ficus lacor*

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 (Chopra *et al.*, 1956).

1.9.1 Taxonomical classification of *Ficus lacor*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Urticales

Family: Moraceae

Genus: *Ficus*

Species: *lacor*



Figure 1.9: *Ficus lacor* Plant

1.9.2 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*, *Ficus macrophylla*, *Ficus microcarpa* (Chinese banyan), *Ficus pseudopalma*, *Ficus pumila*, *Ficus religiosa*, *Ficus saussureana*, *Ficus subulata* etc(Wong, 2007). Ficus species contains alkaloids, flavanoid, steroids, glycosides, phenolic acids , steroids , saponins , coumarins , tannins, triterpinoids etc (Rastogi and Mehrotra, 1970).

1.9.3 Traditional uses

The bark of the *Ficus lacor* Buch - Ham is used traditionally for the treatment of ulcer, expel round worms and for the treatment of leucorrhoea. In skin problems leaves are used (Gamble, 1922; Nadkarni and Nadkarni, 1976). *Ficus lacor* leaves

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reported the presence of various compounds like α - amyrin, β - amyrin, stigmasterol, lupeol and campesterol. The other chemical compounds such as scutellarein, sorbifolin, beraptene, glucoside and bergaptol have been isolated from the whole plant (Jain and Yadav, 1994; Swami and Bist 1996). Various parts i.e. bark, stems, leaves, fruits, roots and aerial roots are used to cure various types of diseases like anticancer, reproductive system, gastrointestinal, respiratory, wound healing, cardiovascular disorders, infectious diseases, anti-arthritis, anti-inflammatory, Central nervous system disorders, antispasmodic and endocrine system diseases (Kirtikar and Basu 1993, Duke *et al.*, 2002, Werbach, 1993, Gurib-Fakim *et al.*, 1996, Kapoor 1990, Diaz *et al.*, 1997, and Duke *et al.*, 1994)

1.9.4 Reported Biological properties of *Ficus* species

1.9.4.1 Anti-microbial activity

The methanol extract isolated from bark of stem of *Ficus ovata* evaluated for the minimal microbicidal concentration against fungi (two species), gram-positive (three species) and gram-negative bacteria (five species). The minimal inhibition concentration determinations indicated that the crude extract was active against 50 percent of the tested microbial species (Kuete *et al.*, 2009).

The aqueous extracts of the *Gossypium arboreum* and *Ficus asperifolia* were tested for antibacterial activity against *S. aureus*, *B. subtilis*, *M. flatus*, *E. coli*, *P. aeruginosa* and SA1199B, XU212 and RN4220. The plant extract effects on fibroblast growth stimulation and antioxidant activity in the same cell line were also calculated. The *Gossypium arboreum* and *Ficus asperifolia* extracts shown less significant antibacterial action against all tested microbes. (Annan *et al.*, 2008).

The methanol extract from the roots of *Ficus polita* and isolated compound 3,5,4'-trihydroxy-stilbene-3,5-O- β -D-diglucopyranoside shown potential for preventing the growth of the 8 tested microbes (Kuete *et al.*, 2011).

Various *Ficus carica* latex extracts viz., methanol, hexane, chloroform and ethyl acetate extracts were screened for their in-vitro antimicrobial potential against five microbial (bacteria) species and 7 strains of fungus. Chloroform and ethyl acetate fractions shown strong microbial inhibition; methanol fraction have a total inhibition against *C. albicans* at the 500 microg/ml concentration and against *C. neoformans* negative effect. *M. canis* strain have been inhibited highly with methanol extract

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approximately 75% and at a conc of 750 micro g/ml, with ethyl acetate extract highly inhibited (Aref *et al.*,2010).

The in vitro antimicrobial activity of the chloroform, methanol and aqueous extracts of *Ficus deltoidea* was checked at concentration of 10, 20 and 50 mg/ml, respectively using the disc diffusion method against two Gram positive strains i.e. *Staphylococcus aureus*, *Bacillus subtilis*, two Gram negative i.e. *E. coli*, *Pseudomonas aeruginosa* and one fungal strain, *Candida albicans* (IMR C-44). All the extracts showed inhibitory activity on the fungus, Gram-positive and Gram-negative bacteria strains tested except for the chloroform and aqueous extracts showed activity only on *B. subtilis*, *E. coli*, and *P. aeruginosa*. (Abdsamah *et al.*,2012).

1.9.4.2 Anti-diabetic activity

Ficus racemosa Linn. fruits 80% aqueous ethanol extract and its water soluble fraction was consistently active in both non-diabetic and types 1 and 2 diabetic model rats when fed simultaneously with glucose load (Jahan *et al.*,2009).

From the stem bark of *Ficus bengalensis* Linn., a dimethoxy derivative of leucocyanidin 3-O-beta-D-galactosyl cellobioside, isolated and screened for anti-diabetic activity. It was reduced blood sugar level significantly in both normal rats group and moderately diabetic rats. It also increased the serum insulin level significant manner in the anon at a dose o250 mg/kg body weight for a 2 h period (Kumar and Augusti 1989).

1.9.4.3 Anti-inflammatory activity

The anti-inflammatory activity of *Ficus religiosa* carried out using five different fractions i.e FR-I, FR-II, FR-III, FR-IV and FR-V, of at the dose level of 20 and 40 mg/kg body weight. The FR-I and FR-III at the dose 40 mg/kg body weight, p.o., were shownen inhibitory effectin cotton pellet granuloma formation, carrageenan induced rat paw edema, and acetic acid induced writhing. (Gulecha *et al.*,2011).

The *Ficus radicans* Roxb. Leaves methanol extract and n-hexane, aqueous methanol and ethyl acetate extracts were investigated for the anti-inflammatory potential. The crude extract and fractions showed a significant inhibition of the inflammation in both models (Naressi *et al.*,2012). The anti-inflammatory and antioxidant activity of *Ficus carica* leaves due to the presence of steroids and flavonoids and its free radical scavenging activity (Ali *et al.*,2012).

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1.9.4.4 Anti HIV

Ficus glomerata wood ethanol extract and its purified components aloe-emodin and 1,3,6-trihydroxy-8-methyl-anthraquinone, at concentrations of 100 microM were investigated for their HIV-1 integrase (IN) and nitric oxide (NO) inhibitory activities. Compound aloe-emodin showed 31.9 percent inhibition of HIV-1 IN, followed by 1,3,6-Trihydroxy-8-methyl-anthraquinone 19.5 percent (Bunluepuech *et al.*, 2011).

1.9.4.5 Hypolipidaemic activity

Ficus mysorensis (hexane extract), was evaluated in vivo for hypolipidaemic activity by estimation of the lipid profile and certain antioxidant parameters in hypercholesterolemic rats. The hexane fraction was chromatographed and six separated compounds were identified. Furthermore, its saponifiable fraction was identified by a MS/MS technique. *Ficus mysorensis* recorded hypolipidaemic and antioxidant effects (Awad *et al.*, 2012).

From the stem bark of *Ficus racemosa* ethanol extract isolated and screened in alloxan-induced diabetic rats. Orally given the extract at the dose 300 mg/kg body weight to alloxan-induced diabetic rats. Extract have significant anti-hypolipidemic and antidiabetic compared with standard drug (Sophia and Manoharan 2007).

Hexane extract of *Ficus microcarpa* leaves have been recorded to show an improvement of lipid profile, antioxidant activity, liver function enzyme stimulation and liver histopathological picture. (Awad *et al.*, 2011).

The water extract isolated from the *Ficus bengalensis* bark and has a significant antioxidant effect, in addition to hypolipidaemic effect in rabbits (Shukla *et al.*, 2004).

1.9.4.6 Cytotoxic activity

The *Ficus carica* hexane-ethyl acetate and hexane extracts isolated from leaves at conc of 78 µg/mL prevent the growth of viruses. There have potent activity for prevention of various diseases spread by adenovirus and echovirus and cytotoxic effect at different concentration on Vero cells not observed (Lazreg *et al.*, 2011).

1.9.4.7 Anti-oxidant activity

Chloroform extract of *Ficus carica* was found to be the richest in total flavonoid content (252.5 +/- 1.1 mg/g quercetin equivalent), while the n-butanol extract had the highest total phenol amount (85.9 +/- 3.2 mg/g extract gallic acid equivalent) (Orhan *et al.*, 2011).

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Ficus glomerata protects tissues from oxidative stress and these effects are probably related to the antioxidant properties (Verma *et al.*, 2010).

The antioxidative activities of water extract and polysaccharide soluble in hot water isolated from fruits of *Ficus carica L.* were evaluated. Both water extract and polysaccharide have notable scavenging activities on DPPH with the EC₅₀ values of 0.72 and 0.61 mg/ml, respectively (Yang *et al.*, 2009).

New megastigmane glycoside, ficalloside, and eleven known compounds, were isolated from methanol extract of *Ficus callosa* leaves and antioxidant activities of these compounds was measured using the oxygen radical absorbance capacity (ORAC) assay. Compound exhibited potent antioxidant activity of 10.6 µM trolox equivalents at the concentration of two µM. (Van *et al.*, 2011). The fatty acid and coumarin content extracted from leaves, wood parts and bark of *Ficus carica*. These were investigated for antioxidant and anti-proliferative potential. The leaves extract shown significant free radical scavenging activity and peroxidation inhibition as compared with wood and bark fraction. The leaves also have anti-proliferative activity with IC₍₅₀₎ values of γ.9β µgm/ml (Conforti *et al.*, 2012).

1.9.4.8 Hepatoprotective activity

Four isolated compounds isolated from leaves methanol extract of *Ficus chlamydocarpa*. Out of four compounds, separated from the methanol extract, alpha-amyrin acetate and luteolin showed a significant hepatoprotective potential, as shown by their capability to inhibit liver cells death and the “lactate dehydrogenase leakage” in carbon tetrachloride intoxication (Donfack *et al.*, 2010).

The methanol extract from *Ficus gnaphalocarpa* (Moraceae) leaves. The phytochemical investigations the six compounds isolated and known as: 3-methoxyquercetin, betulinic acid, catechin, quercetin, quercitrin and epicatechin. The compounds quercitrin, 3-methoxyquercetin and quercetin 5, showing significant hepatoprotective and antioxidant activities (Hubert *et al.*, 2011). The methanol extract isolated from leaves of *Ficus carica* and root bark of *Morus alba* and given at the doses of 50 mg/kg body weight and 150 mg/kg body weight. The extracts of *Morus alba* and *Ficus carica* showed antioxidant and hepatoprotective potential. (Singab *et al.*, 2010). *Ficus racemosa* Linn. (Moraceae) stem bark were studied using petroleum ether (FRPE) and methanol (FRME) extract on model of hepatotoxicity induced by carbon tetrachloride (CCl₄) in rats. Both the extracts resulted in significant decreases in the activities of AST, ALT and ALP, compared to CCl₄ treated rats (Ahmed and

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Urooj 2010). The effect of aqueous extract from *Ficus hirta* on N, N-Dimethylformamide (DMF) induced liver injury in mice. The aqueous extract of *Ficus hirta* has protective effect against DMF-induced acute liver injury in mice (Lv *et al.*,2008).

1.9.4.9 Anti-melanogenic activity

Anti-melanogenic effect of *Ficus deltoidea* extract was tested using cultured melanoma cells (B16F1). The cytotoxicity of the plant extract was calculated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method and find out that the maximum concentration of the extract not shownen cell viability as 0.1%. So we can conclude that *Ficus deltoidea* extract have strapping anti-melanogenic potential that is by inhibition of tyrosinase enzyme action and by genes down-regulation involved in the melanogenesis (Oh *et al.*,2011).

1.9.4.10 Anticonvulsant activity

The aqueous extract of *Ficus religiosa* roots (25, 50 and 100 mg/kg, p.o.) was investigated in strychnine, pentylenetetrazole, picrotoxin and isoniazid-induced seizures in mice. The results showed that an orally given aqueous root extracts of *Ficus religiosa* has dose-dependent and potent anticonvulsant activity against strychnine- and pentylenetetrazole-induced seizures (Patil *et al.*,2011) and Anticonvulsant activity of fig extract (25, 50 and 100mg/kg, i.p.) showed inhibition of the anticonvulsant effect of extract (Singh and Goel 2009; Ahmed *et al.*,2010).

1.9.4.11 Anti-nociceptive activity

The aqueous extract of *Ficus deltoidea* leaves has therapeutically active compounds which possess antinociceptive activity at dose of 100mg/kg body weight, justifying its popular therapeutic use in treating conditions associated with the painful conditions (Sulaiman *et al.*,2008).

1.9.4.12 Anti-ulcer potential

Gastroprotective effect of 50% ethanol extract of *Ficus glomerata* fruit (FGE) was shownen the protection of ulcers in pylorus ligation, cold restraint stress and ethanol induced ulcer. The extract inhibit the oxidative break ageof gastric mucosa by jamming lipid peroxidation and by reduction in superoxide dismutase and increase in catalase activity (Rao *et al.*,2008).

1.9.4.13 Antispasmodic effects

The stem bark methanol extract of *Ficus sycomorus* have been isolated and in phytochemical screening showing the occurrence of saponins, gallic tannins, alkaloids, flavone aglycones and reducing sugars. The extract was tested to its effect on isolated smooth muscle tissues by kymograph for acetylcholine induced contraction. The extract decreased the acetylcholine contaction response of guinea pigs duodena and frogs recti abdominis muscles significant. So its shownen inhibitory activity on muscle contraction (Sandabe *et al.*, 2006). The aqueous-ethanol extract of *Ficus carica* fruits showed antispasmodic effect on the isolated rabbit jejunum preparations and antiplatelet effect using ex vivo model of human platelets and tested positive for alkaloids, flavonoids, coumarins, saponins, sterols and terpenes. (Gilani *et al.*, 2008).

1.9.4.14 Neuroprotective effects

The ethyl acetate extract of stems and fruits of *Opuntia ficus-indica* var. The flavonoids quercetin, quercetin3-methyl ether and (+)-dihydroquercetin were isolated. The quercetin was found to inhibit xanthine oxidase or H₂O⁻² induced oxidative injury in neurons, with expected value IC₍₅₀₎ of 4 to 5 micro gm/ml. the (+)-Dihydroquercetin prevent oxidative neuron injury, but it was less potent than Quercetin (Dok-Go *et al.*, 2003).

1.9.4.15 Central nervous system activity

The methanol extract isolated from stem bark of *Ficus platyphylla* and screened for central nervous system (CNS) activities using mice and rats. The results revealed that the extract significantly reduced the locomotor and exploratory activities in mice, prolonged pentobarbital sleeping time in rats, attenuated amphetamine-induced hyperactivity and apomorphine-induced stereotypy in mice, dose-dependently. The extract significantly suppressed the active-avoidance response in rats, with no significant effect on motor co-ordination as determined by the performance on rotarod (Chindo *et al.*, 2003).

1.9.4.16 Vibridical activity

Four of the seven tested medicinal plants exhibited anti-microbial potential against *Vibrio cholerae*. These seven plants i.e. *Ficus capensis*, *Entada africana*, *Piliostigma reticulatum*, *Mitragyna stipulosa*, *Terminalia avicennoides*, *Lannea acida* and *Terminalia avicennoides* showed antimicrobial activity than others. Potentials of these herbs in the control of cholera need to be determined (Akinsinde and Olukoya 1995).

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1.9.5 Reported phytoconstituents of Ficus species

Table 1.3: List of Reported Phytoconstituents of Ficus species

S. No.	Chemical constituents	Species	Parts	References
1.	Antofine	<i>septica</i>	Leaves	(Baumgartner <i>et al.</i> , 1990; Wu <i>et al.</i> , 2006)
2.	10S,13aR-antofine N-oxide	<i>Septica</i>	Leaves	(Damu <i>et al.</i> , 2005)
3.	Dehydrotylophorine	<i>Septica</i>	Leaves	(Damu <i>et al.</i> , 2005)
4.	2-Demethoxytylophorine	<i>Hispida</i>	Leaves	(Venkatachalam & Mulchandani 1982)
5.	4,6-Bis-(4-methoxy phenyl)-1,2,3-trihydro indolizidinium chloride	<i>Septica</i>	Leaves	(Baumgartner <i>et al.</i> , 1990)
6.	Ficuseptine A	<i>Septica</i>	Leaves	(Wu <i>et al.</i> , 2002)
7.	Ficuseptine B	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
8.	Ficuseptine C	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
9.	Ficuseptine D	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
10.	Hispidine	<i>Hispida</i>	Stems	(Venkatachalam & Mulchandani 1982)
11.	14 α -Hydroxyiso tylocrebrane N-oxide	<i>Septica</i>	Leaves	(Wu <i>et al.</i> , 2002)
12.	Isotylocrebrane	<i>Septica</i>	Leaves & Stems	(Wu <i>et al.</i> , 2002; Damu <i>et al.</i> , 2005)
13.	10S,13aR-isotylocrebrane N-oxide	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
14.	10S,13aS-isotylocrebrane N-oxide	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
15.	6-O-methyltylophorinidine	<i>Hispida</i>	Stems	(Venkatachalam & Mulchandani 1982)
16.	Tylocrebrane	<i>Septica</i>	Leaves & Stems	(Wu <i>et al.</i> , 2002; Damu <i>et al.</i> , 2005)
17.	10R,13aR-tylocrebrane N-oxide	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
18.	10S,13aR-tylocrebrane N-oxide	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
	Tylophorine	<i>Septica</i>	Leaves	(Wu <i>et al.</i> , 2002)
19.	10R,13aR-tylophorine N-oxide	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
20.	10S,13aR-tylophorine N-oxide	<i>Septica</i>	Stems	(Damu <i>et al.</i> , 2005)
21.	Cyanidin-3-O-glucoside	<i>Carica</i>	Fruits	(Solomon <i>et al.</i> , 2006)
22.	Cyanidin-3-O-rhamnoglucoside	<i>Carica</i>	Fruits	(Solomon <i>et al.</i> , 2006)

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S. No.	Chemical constituents	Species	Parts	References
23.	5,3 α -Dimethyl leucocyanidin-3-O- β -galactosyl cellobioside	<i>Benghalensis</i>	Bark	(Daniel <i>et al.</i> ,2003)
24.	5,6-Dimethyl ether of leucopelargonidin-3-O- β -1-rhamnoside	<i>Benghalensis</i>	Bark	(Daniel <i>et al.</i> ,2003)
25.	Bergapten	<i>Carica</i>	Leaves	Innocenti <i>et al.</i> ,1982)
26.	4',5'-Dihydroxyisoflavanone	<i>Carica</i>	Leaves	(Innocenti <i>et al.</i> ,1982)
27.	5,6-O- β -d-diglucopyranosylangelicin	<i>Ruficaulis</i>	Leaves	(Chang <i>et al.</i> ,2005)
28.	Esculin	<i>Septica</i>	Leaves	(Wu <i>et al.</i> ,2002)
29.	5-O- β -d-glucopyranosyl-6-hydroxyangelicin	<i>Septica</i>	Leaves	(Chang <i>et al.</i> ,2005)
30.	6-O- β -d-glucopyranosyl-5-hydroxyangelicin	<i>Ruficaulis</i>	Leaves	(Chang <i>et al.</i> ,2005)
31.	5-O- β -d-glucopyranosyl-8-hydroxypсорален	<i>Ruficaulis</i>	Leaves	(Chang <i>et al.</i> ,2005)
32.	8-O- β -d-glucopyranosyl-5-hydroxypсорален	<i>Ruficaulis</i>	Leaves	(Chang <i>et al.</i> ,2005)
33.	Marmesin	<i>Carica</i>	Leaves	Innocenti <i>et al.</i> ,(1982)
34.	Psoralen	<i>hirta, carica</i>	Leaves & roots	(Innocenti <i>et al.</i> ,1982; Li <i>et al.</i> ,2006)
35.	Umbelliferone	<i>Carica</i>	Leaves	(Wu <i>et al.</i> ,2002)
36.	Alpinumisoflavone	<i>Nymphaeifolia</i>	Leaves	(Darbour <i>et al.</i> ,2007)
37.	Apigenin	<i>Formosana</i>	Stems & roots	Li <i>et al.</i> ,(2006)
38.	Cajanin	<i>Nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
39.	Carpachromene	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
40.	Derrone	<i>Nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
41.	5,7-Dihydroxy-4-methoxy-3'-(2,3-dihydroxy-3-methylbutyl)isoflavone	<i>Nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
42.	Erycibenin A	<i>Nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
43.	Erycibenin C	<i>Nymphaeifolia</i>	Stems	(Darbour <i>et al.</i> ,2007)
44.	Ficusflavone	<i>Microcarpa</i>	Bark	Li & Kuo (1997)
45.	Genistein	<i>Nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
46.	Genistin		Leaves	(Wu <i>et al.</i> ,2002)
47.	Hesperidin	<i>Hirta</i>	Roots	(Li <i>et al.</i> ,2006)
48.	5-Hydroxy-4',6,7,8-tetramethoxyflavone	<i>Hirta</i>	Roots	(Li <i>et al.</i> ,2006)

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S. No.	Chemical constituents	Species	Parts	References
49.	Isoglabranin)	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
50.	Isolupinisoflavone E	<i>Microcarpaf</i>	Bark	Li & Kuo (1997)
51.	Isoquercitrin	<i>Septica</i>	Leaves	(Chang <i>et al.</i> ,2005)
52.	Kaempferitin	<i>Carica</i>	Leaves	(Wu <i>et al.</i> ,2002)
53.	3_-(3-Methylbut-2-enyl)biochanin A	<i>nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
54.	Norartocarpanone	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
55.	Norartocarpentin	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
56.	4,5,6,7,8-Pentamethoxyflavone	<i>Hirta</i>	Roots	(Li <i>et al.</i> ,2006)
57.	Rutin	<i>Septic</i>	Leaves	(El-Kholy &Shaban 1966; Chang <i>et al.</i> ,2005)
58.	5,7,2'-Trihydroxy-4'-methoxyisoflavone	<i>nymphaeifolia</i>	Stems bark	(Darbour <i>et al.</i> ,2007)
59.	5-Acetyl-2-hydroxyphenyl- β -d-glucopyranoside	<i>Septic</i>	Leaves	(Wu <i>et al.</i> ,2002)
60.	Bergenin	<i>Racemosa</i>	Bark	(Li <i>et al.</i> ,2004)
61.	trans-4,5-Bis(4-hydroxy-3-methoxyphenyl)-1,3-dioxacyclohexane	<i>Beecheyana</i>	Roots	(Lee <i>et al.</i> ,2002)
62.	erythro-2,3-Bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol	<i>Beecheyana</i>	Roots	(Lee <i>et al.</i> ,2002)
63.	threo-2,3-Bis(4-hydroxy-3-methoxyphenyl)-3-ethoxypropan-1-ol	<i>Beecheyana</i>	Roots	(Lee <i>et al.</i> ,2002)
64.	2,3-Dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone	<i>Beecheyana</i>	Roots	(Lee <i>et al.</i> ,2002)
65.	Ficuformodiol A	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
66.	Ficuformodiol B	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
67.	threo-3-(4-Hydroxy-3,5-dimethoxyphenyl)-3-ethoxypropane-1,2-diol	<i>Beecheyana</i>	Roots	(Lee <i>et al.</i> ,2002)
68.	3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)	<i>Beecheyana</i>	Roots	(Lee <i>et al.</i> ,2002)
69.	(R)-(-)-Mellein	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)

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S. No.	Chemical constituents	Species	Parts	References
70.	3-(7-Methoxy-2,2-dimethyl-2H-6-chromenyl)-(E)-propenoic acid	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
71.	Obovatin	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
72.	Racemosic acid	<i>Racemosa</i>	Bark	(Li <i>et al.</i> ,2004)
73.	Spatheliachromene	<i>Formosana</i>	Stems	(Sheu <i>et al.</i> ,2005)
74.	Vanillic acid	<i>Septica</i>	Leaves	(Wu <i>et al.</i> ,2002)
75.	Uracil	<i>Septica</i>	Leaves	(Wu <i>et al.</i> ,2002)
76.	3 β -Acetoxy- α -amyrin	<i>Hirta</i>	Roots	(Li <i>et al.</i> ,2006)
77.	3 β -Acetoxy- β -amyrin	<i>Hirta</i>	Roots	(Li <i>et al.</i> ,2006)
78.	3 β -Acetoxy-12,19-dioxo-13(18)-oleanene	<i>Microcarpa</i>	Aerial roots	(Chang <i>et al.</i> ,2005)
79.	3 β -Acetoxy-1 β ,11 α -epidioxy-12-ursene	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2001)
80.	3 β -Acetoxy-12 β ,13 β -epoxy-11 α -hydroperoxyursane	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2001)
81.	3 β -Acetoxy-11 α ,12 α -epoxy-16-oxo-14-taraxerene	<i>Microcarpa</i>	Aerial roots	(Chang <i>et al.</i> ,2005)
82.	3 β -Acetoxy-11 α ,12 α -epoxy-14-taraxerene	<i>Microcarpa</i>	Aerial roots	(Chang <i>et al.</i> ,2005)
83.	3 β -Acetoxy-20 α ,21 α -epoxytaraxastane	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2001)
84.	3 β -Acetoxy-21 α ,22 α -epoxytaraxastan-20 α -ol	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> ,2005)
85.	3 β -Acetoxy-20 α ,21 α -epoxytaraxastan-22 α -ol	<i>Microcarpa</i>	Leaves	(Chiang &Kuo 2000)
86.	3 β -Acetoxy-11 α -hydroperoxy-13 α -h-ursan-12-one	<i>Microcarpa</i>	Aerial roots	Chiang &Kuo (2000)
87.	(20S)-3 β -acetoxyl-20-hydroperoxy-30-norlupane	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2001)
88.	3 β -Acetoxy-18 α -hydroperoxy-12-oleanen-11-one	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2001)
89.	3 β -Acetoxy-19 α -hydroperoxy-20-taraxastene	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2000)
90.	3 β -Acetoxy-11 α -hydroxy-11-(12 \rightarrow 13)abeooleanan-12-al	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2002)

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S. No.	Chemical constituents	Species	Parts	References
91.	3 β -Acetoxy-25-hydroxylanosta-8,23-diene	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> , 2005)
92.	(20S)-3 β -Acetoxylupan-29-oic acid	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2001)
93.	3 β -Acetoxy-25-methoxylanosta-8,23-diene	<i>Microcarpa</i>	Aerial roots	(Chang <i>et al.</i> , 2005)
94.	3 β -Acetoxy-19 α -methoxy-20-tanaxastene	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2000)
95.	3 β -Acetoxy-22 α -methoxy-20-tanaxastene	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2000)
96.	3 β -Acetoxyolean-12-en-11 α -ol	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2000)
97.	3 β -Acetoxy-12-oleanen-11-one	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2000)
98.	3 β -Acetoxy-19(29)-taraxasten-20 α -ol	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> , 2005)
99.	3 β -Acetoxy-20-tanaxasten-22 α -ol	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2000)
100.	Acetylbetulinic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> , 2005)
101.	Acetylursolic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> , 2005)
102.	Baurenol	<i>Carica</i>	Leaves	(Ahmed <i>et al.</i> , 1988)
103.	Betulonic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> , 2005)
104.	29,30-Dinor-3 β -acetoxy-18,19-dioxo-18,19-secolupane	<i>Microcarpa</i>	Aerial roots	(Chiang & Kuo 2002)
105.	3,22-Dioxo-20-tanaxastene	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> , 2005)

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S. No.	Chemical constituents	Species	Parts	References
106.	3 β -Hydroxy-20-oxo-29(20→19)abeolupane	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2002)
107.	3 β -Hydroxy-stigmast-5-en-7-one	<i>Hirta</i>	Roots	(Li <i>et al.</i> ,2006)
108.	6-O-linoleyl- β -d-glucosyl- β -sitosterol	<i>Carica</i>	Latex	(Rubnov <i>et al.</i> ,2001)
109.	Lupenol	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2002)
110.	Lupenol acetate	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2002)
111.	Lupeol	<i>Carica</i>	Leaves	(Saeed &Sabir 2002)
112.	24-Methylenecycloartanol	<i>Carica</i>	Leaves	(Ahmed <i>et al.</i> ,1988)
113.	Oleanonic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> ,2005)
114.	6-O-oleyl- β -d-glucosyl- β -sitosterol	<i>Carica</i>	Latex	(Rubnov <i>et al.</i> ,2001)
115.	3-Oxofriedelan-28-oic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> ,2005)
116.	6-O-palmitoyl- β -d-glucosyl- β -sitosterol	<i>Carica</i>	Latex	(Rubnov <i>et al.</i> ,2001)
117.	β -Sitosterol	<i>hirta & carica</i>	Leaves &roots	(El-Kholy &Shaban 1966; Wu <i>et al.</i> ,2002; Li <i>et al.</i> ,2006)
118.	β -Sitosterol- β -d-glucoside	<i>Septic</i>	Leaves	(Wu <i>et al.</i> ,2002)
119.	6-O-stearyl- β -d-glucosyl- β -sitosterol	<i>Carica</i>	Latex	(Rubnov <i>et al.</i> ,2001)
120.	Stigmasterol	<i>hirta & septic</i>	Leaves &roots	(Wu <i>et al.</i> ,2002; Li <i>et al.</i> ,2006)
121.	Squalene	<i>Septic</i>	Leaves	(Wu <i>et al.</i> ,2002)
122.	20-Taxastene-3 β ,22 β -diol	<i>Microcarpa</i>	Aerial roots	(Chiang &Kuo 2000)

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S. No.	Chemical constituents	Species	Parts	References
123.	β -Taraxasterol ester	<i>Septic</i>	Leaves	(El-Kholy &Shaban 1966)
124.	Ursolic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> ,2005)
125.	Ursonic acid	<i>Microcarpa</i>	Aerial roots	(Chiang <i>et al.</i> ,2005)
126.	3,4,5-Trihydroxydehydro- β -ionol-9-O- β -d-glucopyranoside	<i>Septic</i>	Leaves	(El-Kholy &Shaban 1966)
127.	Ficusamide	<i>Elastic</i>	Aerial roots bark	(Mbosso <i>et al.</i> ,2012)
128.	Ficusoside	<i>Elastic</i>	Aerial roots bark	(Mbosso <i>et al.</i> ,2012)
129.	Elasticoside	<i>Elastic</i>	Aerial roots bark	(Mbosso <i>et al.</i> ,2012)

INTRODUCTION

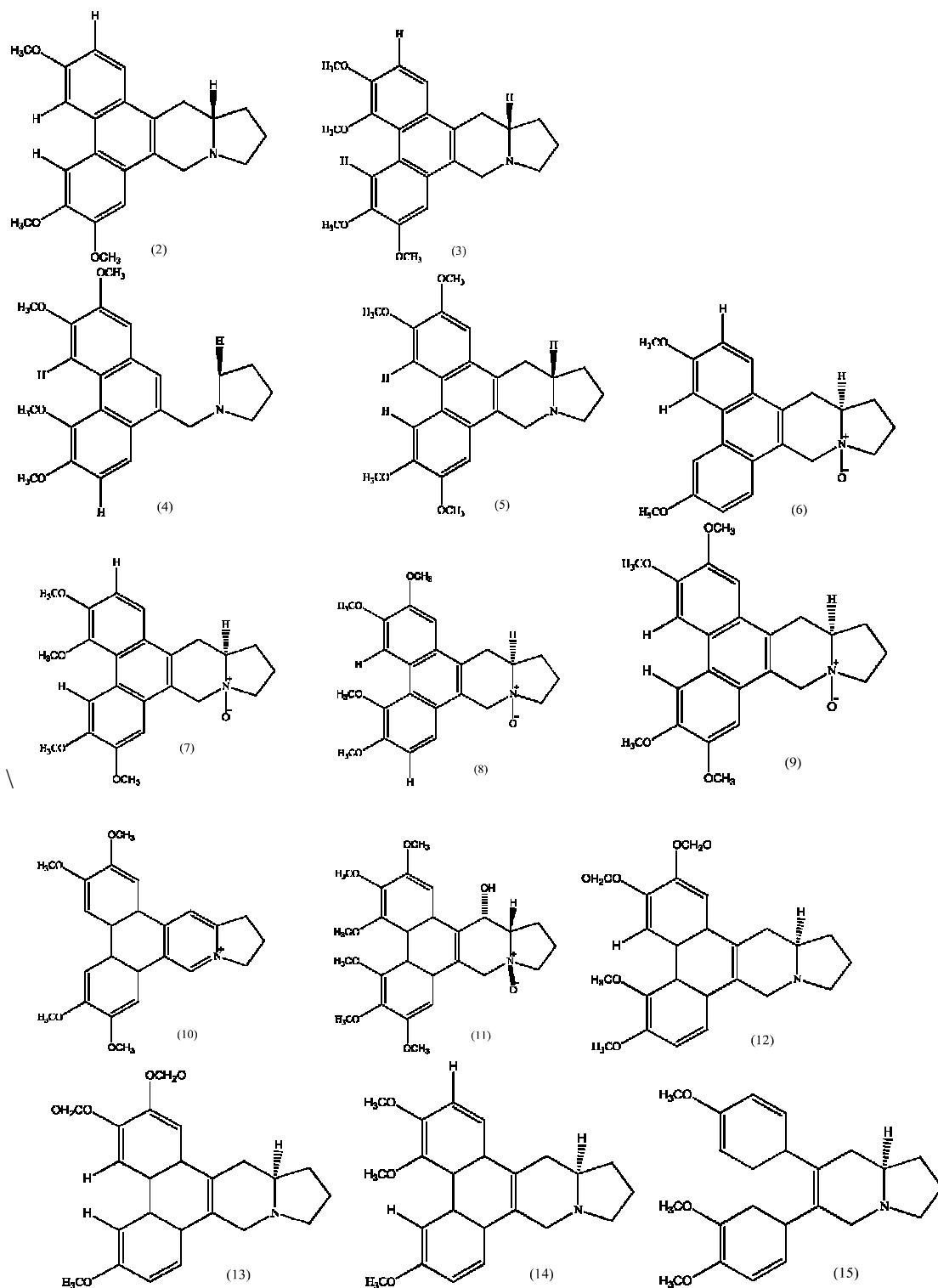


Figure 1.10: Reported phytoconstituents of *Ficus* species

INTRODUCTION

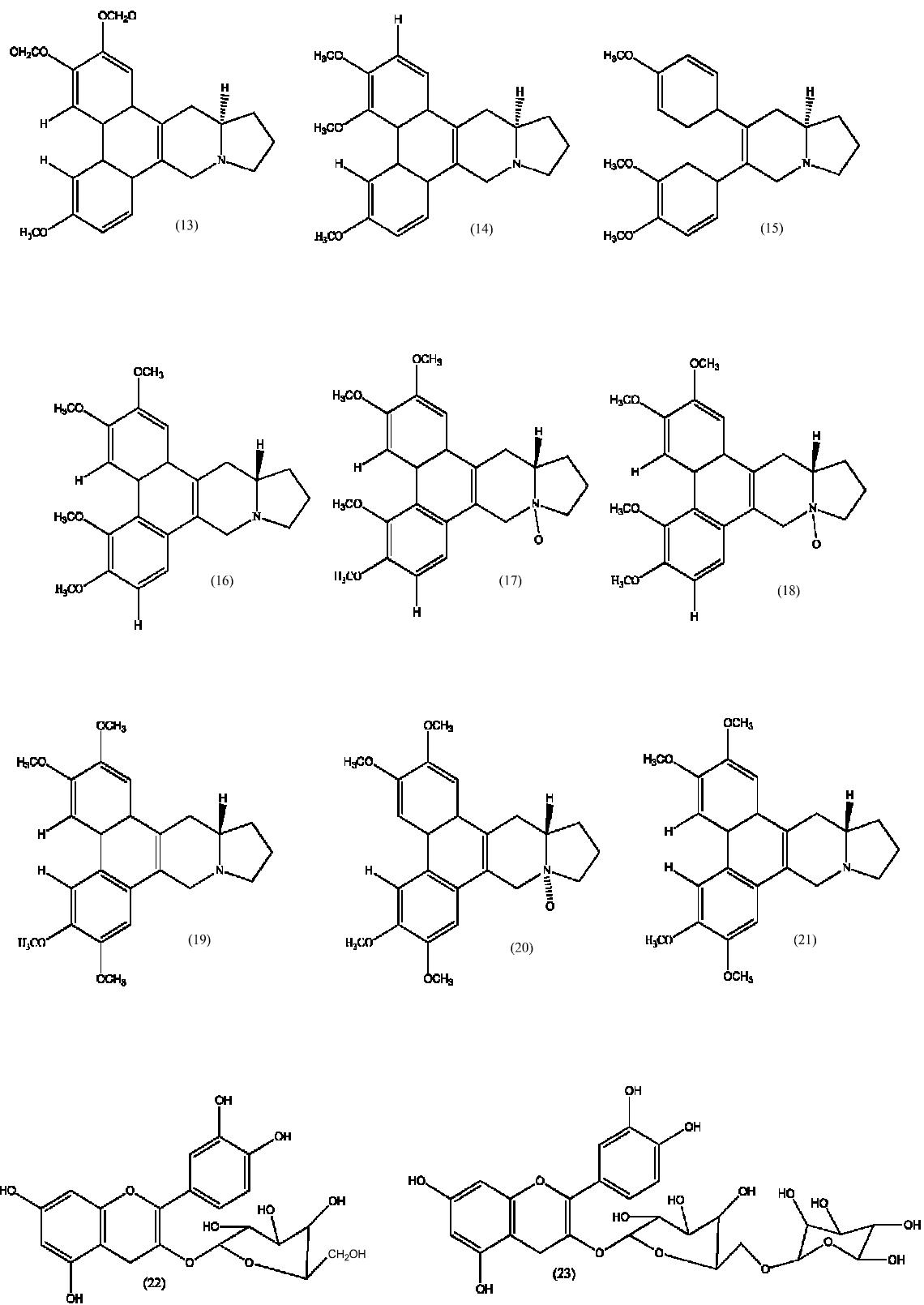


Figure 1.11: Reported phytoconstituents of *Ficus* species

INTRODUCTION

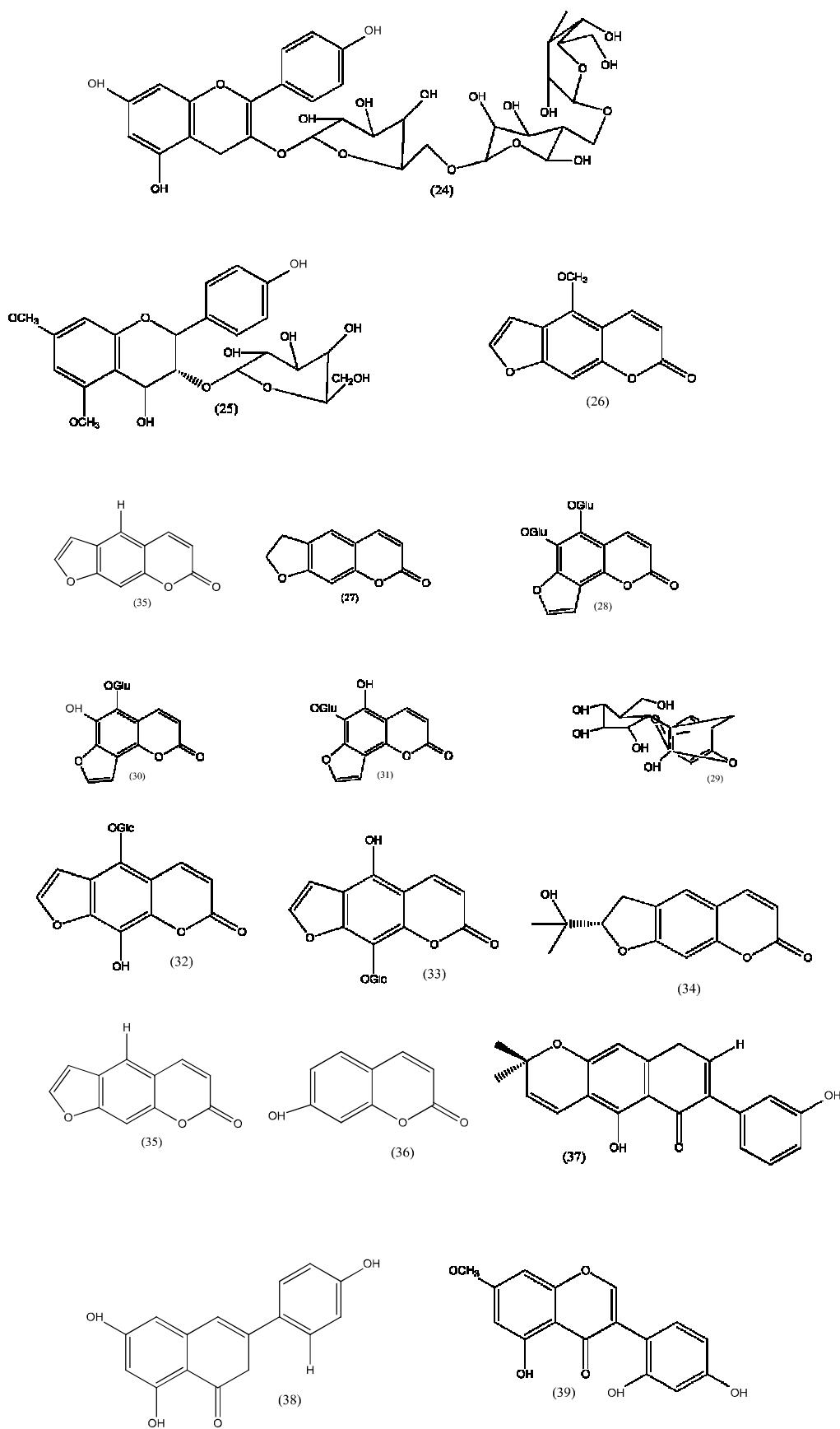


Figure 1.12: Reported phytoconstituents of *Ficus* species

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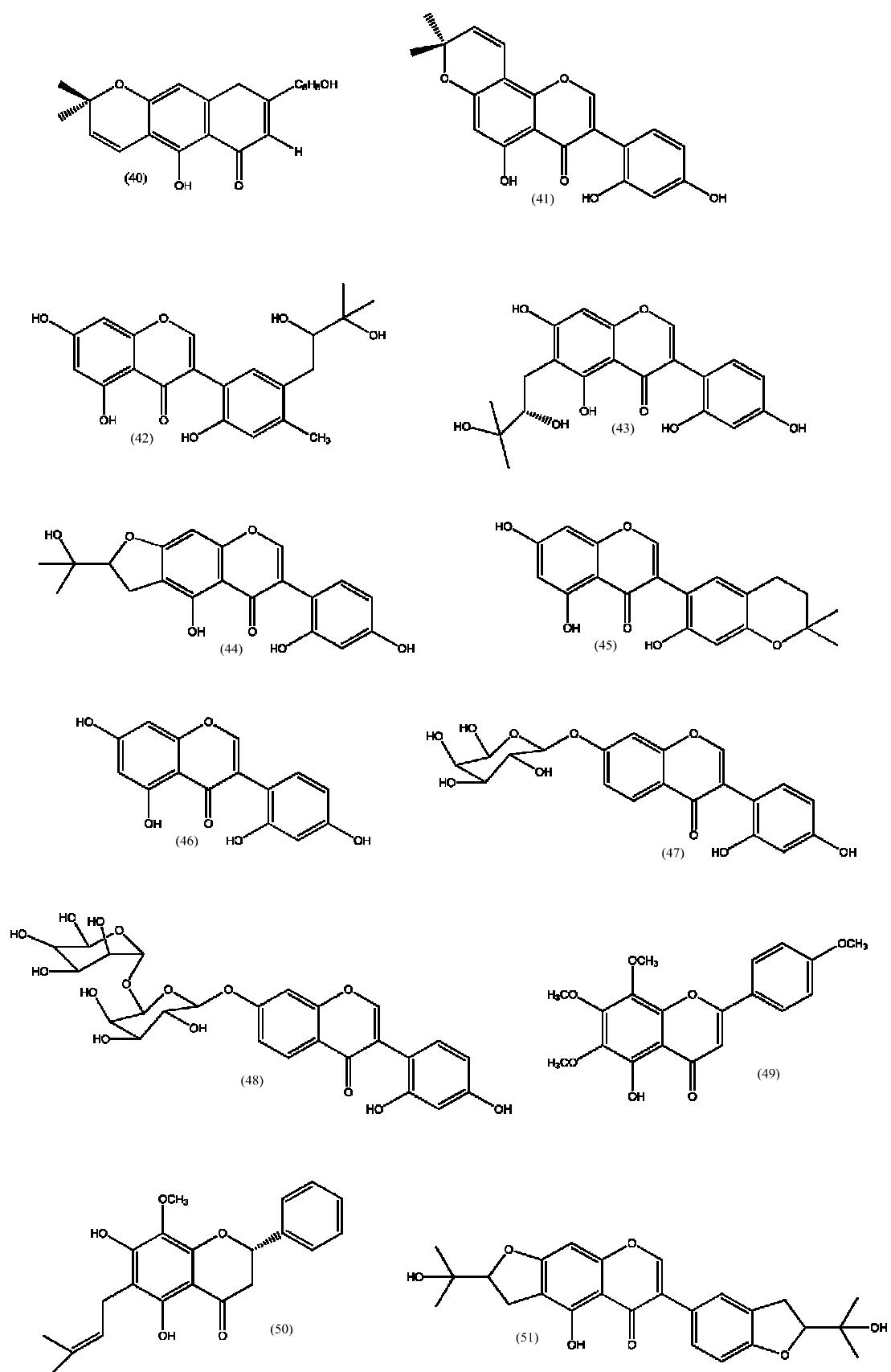


Figure 1.13: Reported phytoconstituents of *Ficus* species

INTRODUCTION

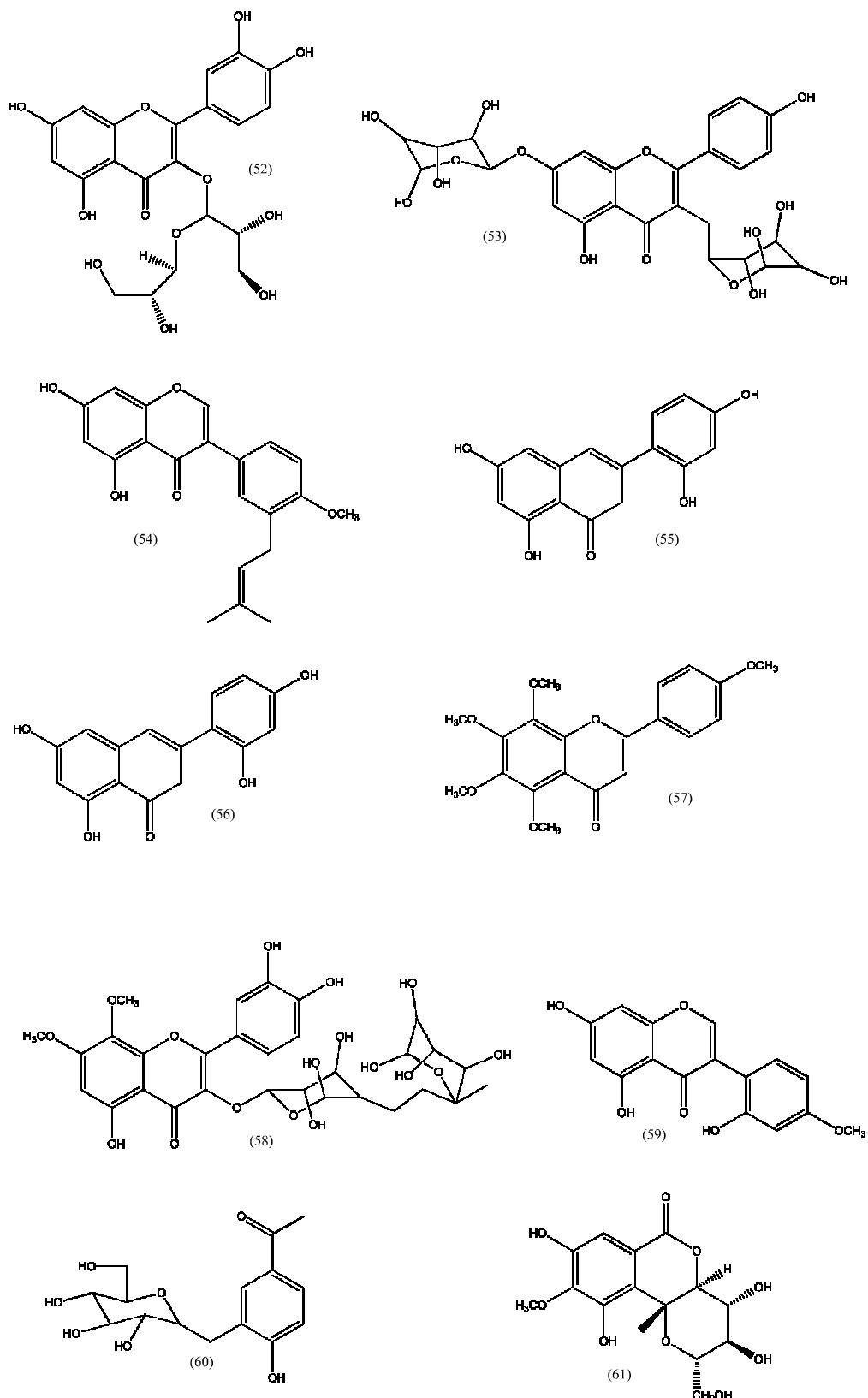


Figure 1.14: Reported Phytoconstituents of *Ficus* Species

INTRODUCTION

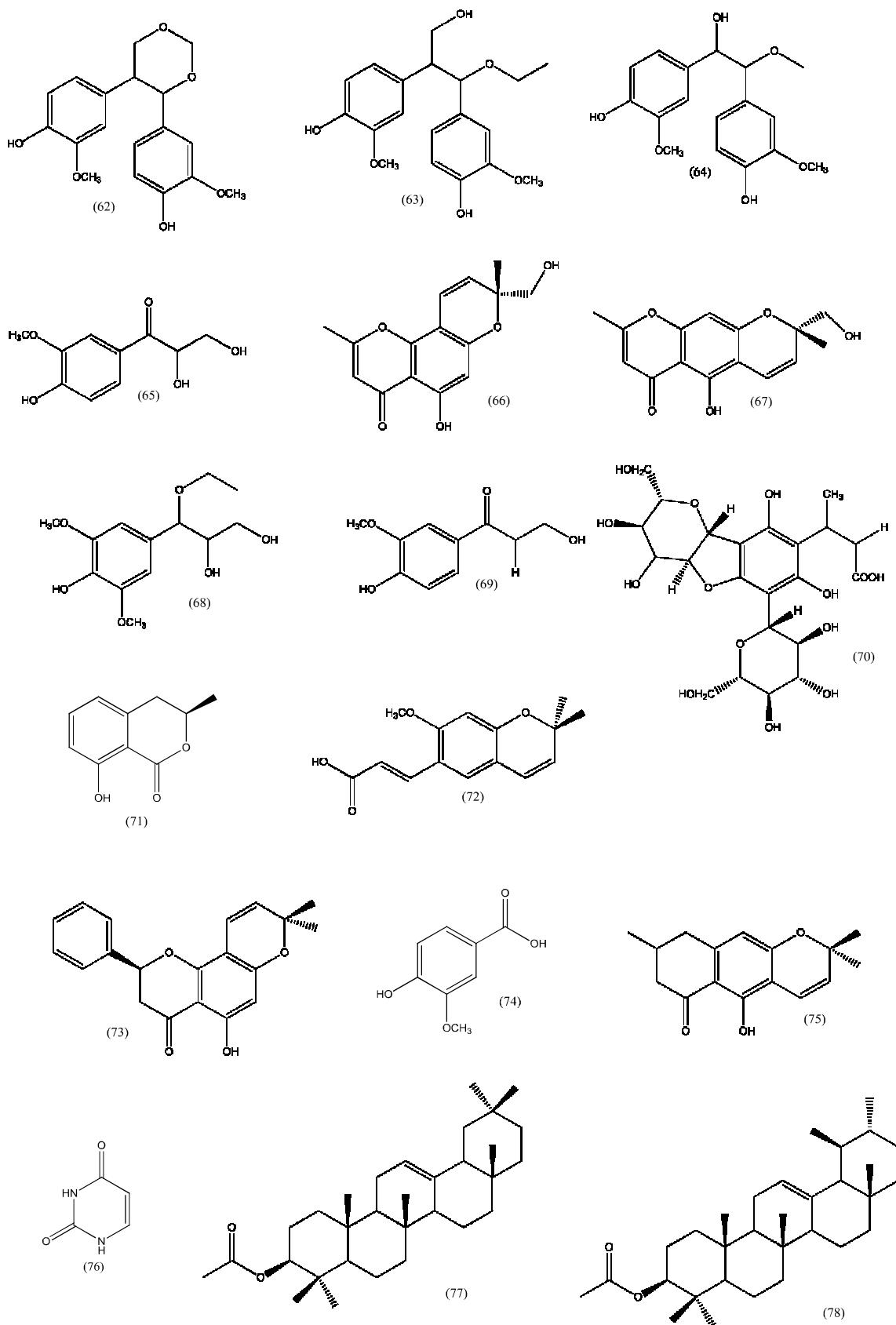


Figure 1.15: Reported Phytoconstituents of *Ficus* Species

INTRODUCTION

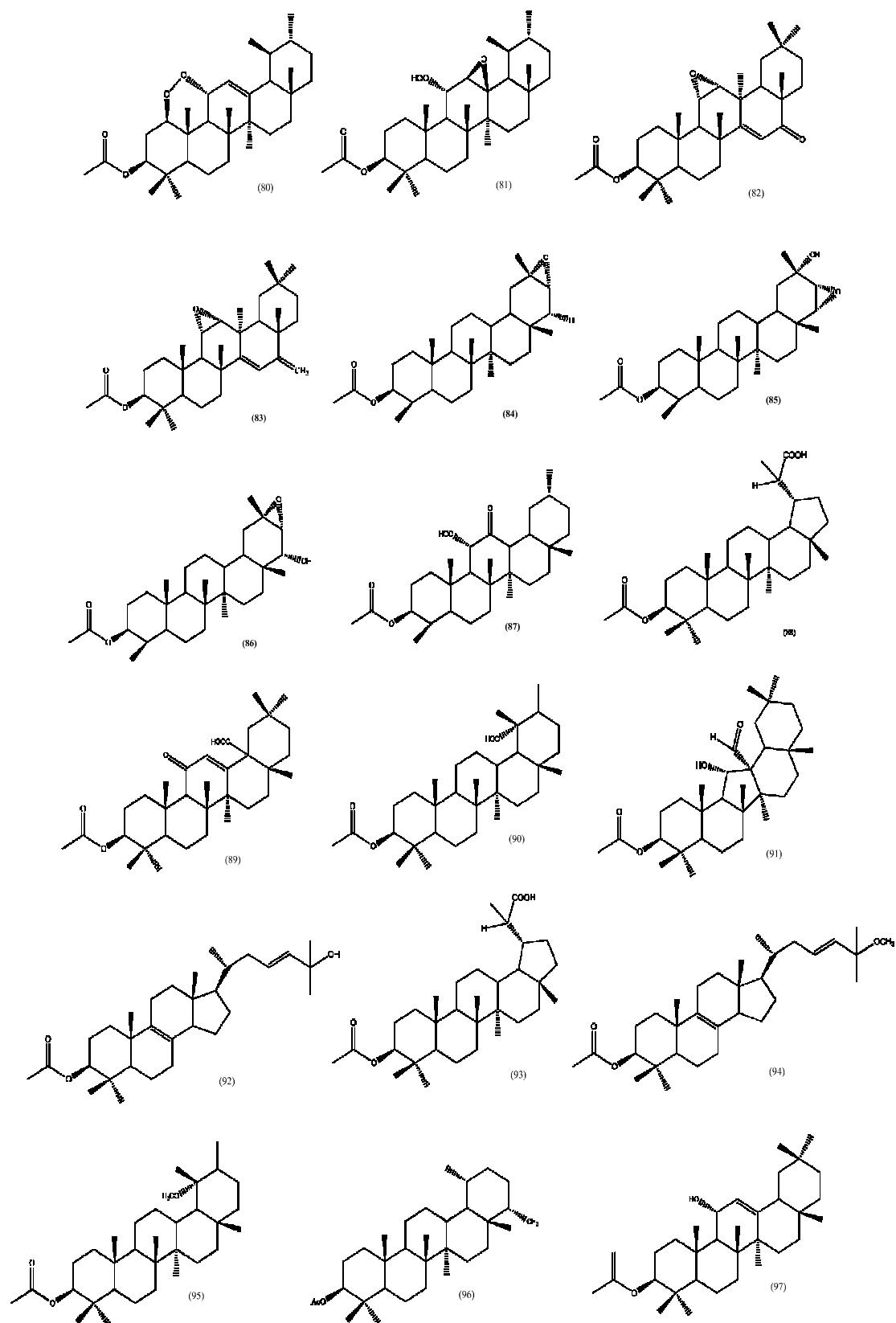


Figure 1.16: Reported Phytoconstituents of Ficus Species

INTRODUCTION

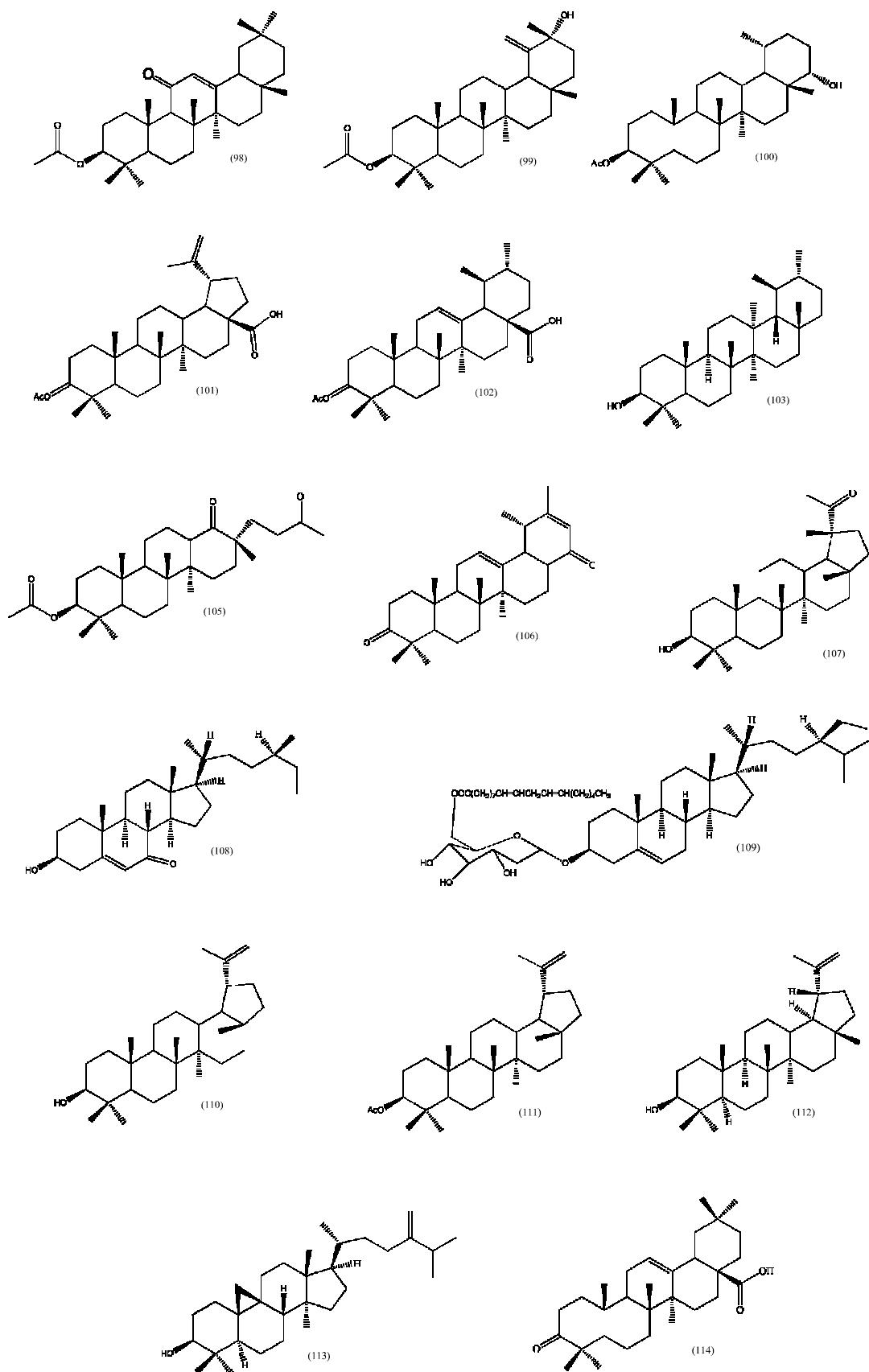


Figure 1.17 Reported Phytoconstituents of Ficus Species

INTRODUCTION

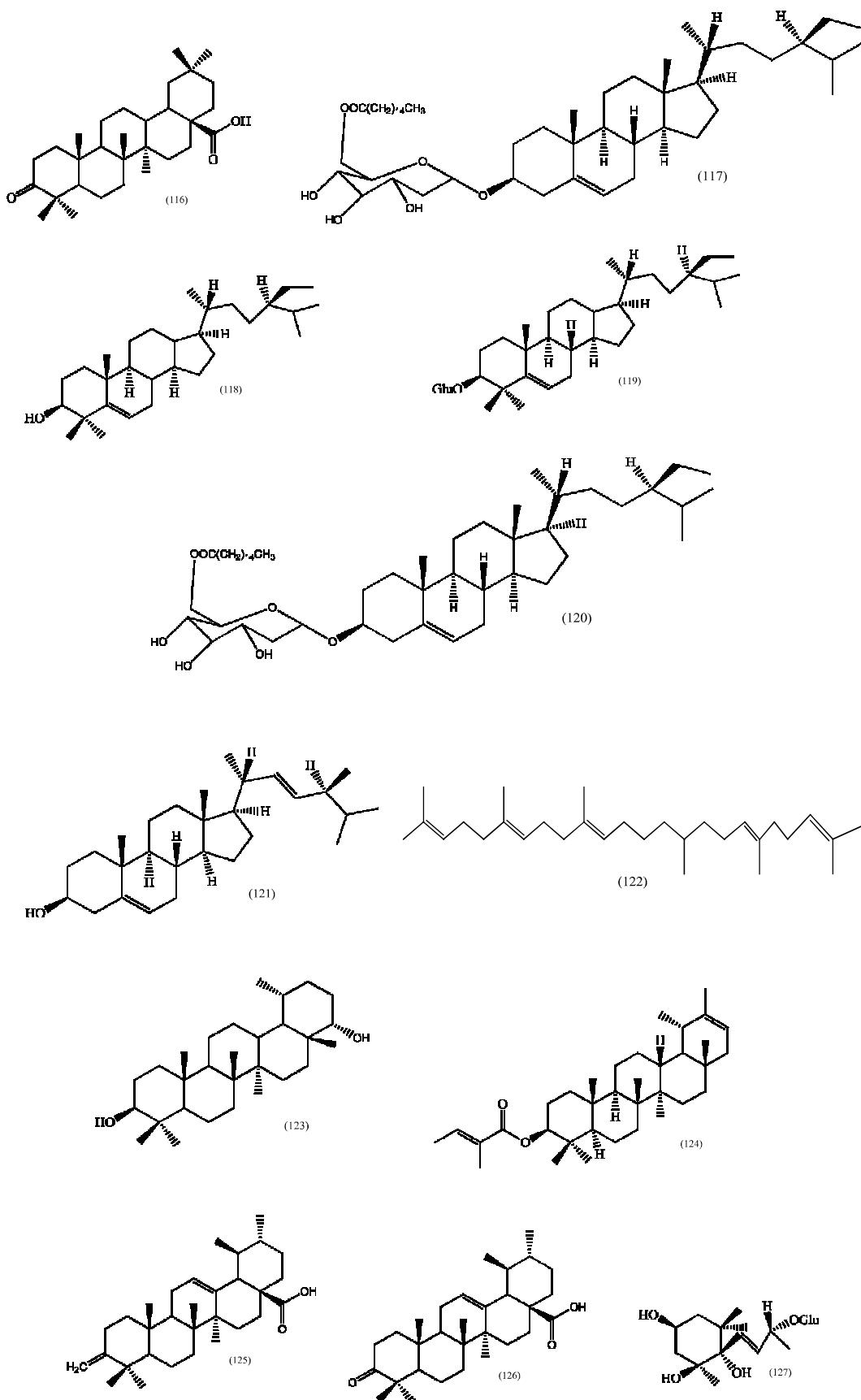
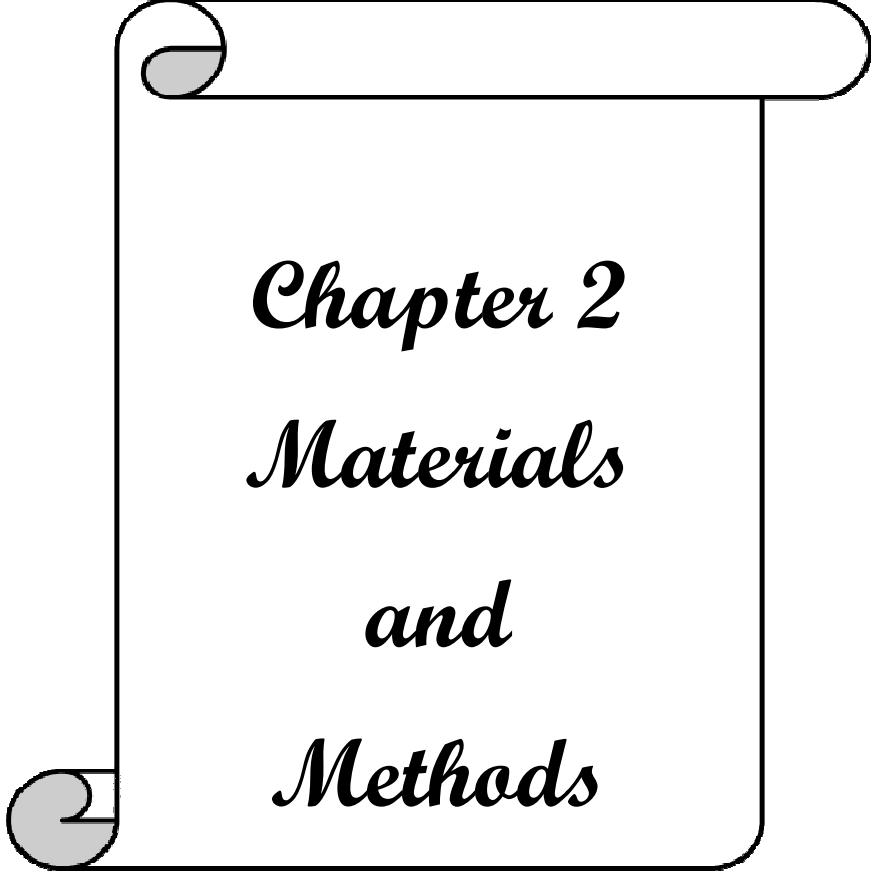


Figure 1.18: Reported Phytoconstituents of *Ficus* Species



Chapter 2
Materials
and
Methods

2.1 Collection of Plant Material

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, in July 2009. The taxonomically, authenticated and identified plant material by Dr. H.B. Singh, HRMHM department, with reference number NISCAIR/RHMD/Consult/2010-11/1638/236. 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.

2.2 Materials

2.2.1 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. The animals (weighing 160-200g) were house in standard environmental conditions ($25\pm2^{\circ}\text{C}$ and rel. humidity $50\pm5\%$) and fed with standard go on a diet and water *ad libitum*. The rats were destitute of food for 24 hrs before conducting tests but allowed free access to tap water throughout the experiment. Each group comprises 6 rats.

2.2.2. 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, aniisaldehyde, α -naphthol, bismuth carbonate, calcium chloride, copper sulphate, Ferric chloride, Follin's reagent, Iodine, Lead acetate, Magnesium chloride, Mercuric chloride, Ninhydrin, Nitric acid, Phloroglucinol, Potassium iodide, Potassium Dichromate, Potassium sodium Tartarate, Ruthenium red, Safranine, Sodium acetate, Sodium iodide, Sodium hydroxide, Sodium nitroprusside, Carrageenan (Hi-Media), DPPH, Sudan red- III. All the chemicals (S.D Fine Chemicals Pvt. Ltd. Mumbai) were purchased from local supplier.

Solvents: Acetic acid, Acetone, Benzene, n-Butanol, Chloroform, Dichloromethane, Ethanol, n-Hexane, Methanol, Pyridine, Petroleum ether, Tween-80, Toluene, and Xylene. All the solvents (S.D. Fine chemicals) were purchased.

2.2.3. INSTRUMENTS:

1. Double beam UV-VIS Spectrophotometer, 2201(Systronics)
2. FT-IR (SHIMADZU)
3. Buchi rottap evaporator
4. UV-Chamber
5. BOD Incubator
6. Moisture balance
7. Microtome
8. Plethysmometer
9. Microscope with camera

2.3 Pharmacognostic studies

India has a rich traditional systems of medicines which is mainly consist of the highly promising systems of thearpies *i.e.* Ayurvedic, Siddha & Unani systems etc. since ancient times. The plant crude drugs are available easily in abundance, they are comparatively cheaper. They have negligible side effects and are commonly prescribed to all age groups patients. The pharmacological actions and uses of herbal drugs are explained in the classical literature of home-grown medicines in so many books of medicinal plant and pharmacopoeias (Chopra, 1955 and Nandkarni, 2000).

The pharmacognostical evaluation is the prelude stride in the standardization of plant drugs. It gives very important information about the morphology, microscopic and physical properties of herbal drug. The observations obtained from standardization, had been included as monographs in various pharmacopoeias. Therefore pharmacognostic studies gives the scientifically important information rabout the indenty, purity and quality of the plant drugs (Thomas *et al.*, 2008).

There is no information in the literature regarding the pharmacognostical evaluation of aerial roots of *Ficus lacor* and roots of *Murraya koenigii*. The present study includes study of morphology, microscopy, powder study, determination of ash values, extractive value, bitterness value, haemolytic activity, microbial determination. It also includes phytochemical screening and chromatographic study of aerial roots of *Ficus lacor* and roots of *Murraya koenigii*.

2.3.1 Morphological study

The crude drug was evaluated for organoleptic properties shape, size, colour, odour, taste, fracture and texture .

2.3.2 Microscopic study

Microscopy of plant material is performed to distinguish it from the allied drugs and adulterant. The dried root was soaked overnight in water to make it smooth enough for transverse section. Paraffin wax embedded specimens were sectioned using the rotatory microtome (**Weswox Optik**). The thickness of section was 10-12 μm . Very fine section was selectively subjected to staining reaction with staining reagent safranin one precent solution and light green 0.2% solution. Slides were cleaned in xylol and mounted in mountant (DPX). Photomicrographs were taken using trinocular microscope (Olympus (Johnsen, 1940).

2.3.3 Histochemical Colour Reaction

Presence of different organic compounds in root of the plant is confirmed by using various histochemical tests. Care was taken to ascertain relative concentration of these chemicals by degree of colour produced in different tissues. The transverse sections of fresh root were treated with different chemical reagents for colour tests viz. phloroglucinol, millon's reagent, iodine solution followed by sulphuric acid, Dragendroff's reagent, Wagner's reagent, Sulphuric acid solution, Libberman-Burchard reagent, Acetic anhydride, ferric chloride, Iodine solution, Caustic alkali, Aqueous potassium hydroxide, Chloroform with sulphuric acid, aniline sulphate and sulphuric acid (Govil *et al.*, 1993).

2.3.4 Powder studies

2.3.4.1 Microscopic study

The shade dried roots were mechanically pulverized to coarse powder and sifted through 40 mesh sieve. Take a pinch of powder was taken on slide and mounted with phloroglucinol, hydrochloric acid and glycerine. Slide was seen under microscope (Evans, 1996).

2.3.4.2 Colour reactions

To study the behaviour of root powder with different chemical reagents, a small quantity of powder was treated with different chemical reagents as 1N hydrochloric acid, sodium hydroxide, acetic acid, 5% ferric chloride, picric acid, nitric acid with ammonia solution, 5% iodine, 1N nitric acid and powder as such were performed, change in colour was observed (Hashmi *et al.*, 2003).

2.3.4.3 Fluorescence behaviour of powder

Many herbs show fluorescence behaviour when cut surface or powder is exposed to UV light and this can help in their identification. To study the fluorescence nature of root powder, powder was treated with different chemical reagents *viz.* 1N sodium hydroxide, 1N hydrochloric acid, 1N sodium hydroxide in methanol, picric acid, 1N nitric acid, acetic acid, acetone, 50% sulphuric acid, nitric acid in ammonia solution and observed under day light, long UV (365 nm) and short UV light (254 nm) (Kokashi *et al.*, 1958).

2.3.5 Ash Values (Anonymous, 1996)

2.3.5.1 Total ash

Total ash is produced by incinerating the drug at the temperature possible to remove all of the carbon. A higher temperature may result in the changes carbonates to oxides. Total ash generally consists of phosphates, carbonates, silica and silicates which includes both physiological ash and non-physiological ash. *e.g.*, sand and soil. About 2 g of air-dried powdered drug was accurately weighed and taken in a silica crucible and incinerate at a temperature not more than 450°C until free from carbon. The crucible was cooled, weighed and %age of total ash was evaluated with reference to the dried drug.

2.3.5.2 Water soluble ash

Water-soluble ash is that part of the total ash portion which was soluble in water. Then total ash obtained was boiled, about 5 minutes with 25 ml of water and insoluble material was collected in an ashless filter paper, incinerated at a temperature not exceeding 450°C, subtracted the weight of the insoluble substance from the weight of the ash and computed the rate of water soluble ash amid reference to the dried drug.

2.3.5.3 Acid-insoluble ash

The acid insoluble ash is calculated by treating, the total ash with dilute HCl and weighing the residue. This limit particularly indicating contamination with siliceous materials such as earth and sand by comparison with the total ash value for the same sample differentiation can be made between contaminating material and in the natural ash of the drug. The total ash obtained, by boiling with 25 ml of 2 N HCl for 5 min, the insoluble matter was collected in an ashless filter paper, wash with boiled water, ignited, cooled in dessicator and weigh. The proportion of acid-insoluble ash with reference to the dried drug was evaluated.

2.3.5.4 Sulphated ash

1 gm of air dried powder drug was treated with dilute sulphuric acid before ignition in a tared silica crucible to a constant weight. The ash obtained was weighed. The percentage of sulphated ash was intended with reference to the dried drug.

2.3.6 Extractive Value (Anonymous, 1996)

Extractive value is used as a evaluating crude drug which are not readily estimated by other means. It is employed for that material for which no suitable chemical or biological assay method exist.

2.3.6.1 Petroleum ether extractive

Accurately weighed 5 gm of the air dried powdered plant material was soaked in 100 ml of pet. ether (60-80°), in a stoppered flask for 24 hrs. The mixture was vigorously shaken at regular intervals. After 24 hrs the solution was filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and also, weighed. The rate of pet. ether solvent extractive was computed with reference to air dried plant material.

2.3.6.2 Chloroform extractive

Accurately weighed 5 gm of the powdered plant drug was macerated by 100 ml of chloroform in a stoppered flask for 24 hrs. The mixture was vigorously shaken at regular intervals. After 24 hrs the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The rate of chloroform solvent extractive was computed with reference to air dried plant material.

2.3.6.3 Ethyl acetate extractive

Accurately weighed 5 gm of the powdered plant drug was macerated through 100 ml of ethyl acetate in a stoppered flask for 24 hrs. The mixture was vigorously shaken at regular intervals. After 24 hrs solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The rate of ethyl acetate solvent extractive was computed with reference to air dried plant material.

2.3.6.4 Ethanol extractive

Accurately weighed 5 gm of the powdered plant drug was macerated by 100 ml of ethanol in a stoppered flask for 24 hrs. The mixture was vigorously shaken at regular intervals. After 24 hrs the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The rate of ethanol solvent extractive was computed with reference to air dried plant material.

2.3.6.5 Water extractive

Accurately weighed 5 gm of dried powdered plant drug was macerated amid 100 ml of water in a stoppered flask for 24 hrs. The mixture was vigorously shaken at regular intervals. After 24 hrs the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weigh. The percentage of aqueous soluble extract was calculated with reference to dried plant material.

2.3.7 Determination of Crude Fiber Content

2 gm of powdered drug extracted with diethyl ether and added 200 ml of boiling dilute sulphuric acid (1.25%) to the ether exhausted marc in a 500 ml flask. The mixture was refluxed for 30 min, filtered through filter paper and the residue was washed with boiling water until the effluent washing was acid free. Rinsed the residue and placed back into the flask with 200 ml of boiling sodium hydroxide solution (1.25%) and refluxed the mixture again for 30 min., filtered through ashless filter paper and washed the residue with boiling water until the last washing was neutral. It was then dried at 110°C to constant weight and then ignited to constant weight. The ash was cooled in dessicator, weighed and calculated as follows (Knevl and Digangi, 1977).

$$\text{Percentage of Crude Fibre} = \frac{\text{Weight of the ash obtained}}{\text{Weight of the drug sample}} \times 100$$

2.3.8 Loss on Drying

This parameter is used to determine the amount of moisture present in a particular sample. The powder (2 gm) sample was placed on a tared evaporating dish. The evaporating dish was dried at $105 \pm 1^\circ\text{C}$ until constant weight and weighed. The drying was continued until two successive readings match each other (Anonymous, 1996).

2.3.9 Determination of Swelling Index (WHO, 1998)

Swelling properties of medicinal plants shows specific therapeutic utility e.g. gums, pectin, or hemicellulose. One g of plant material was accurately weighed, placed into 25 ml glass stoppered measuring cylinder. 25 ml water was added and shaken the mixture thoroughly in every 10 min for one hr and allowed stand for 3 hrs at room temperature. Measured the volume in ml occupied by plant material and calculated the mean value of individual determination, related to one gm of crude plant material.

2.3.10 Foaming Index Determination (Anonymous, 1996)

The medicinal plant materials contain saponins that cause the persistent foam formation when a water decoction is stunned. The foam forming capability of plant material and their extract is measured in term of foaming index. 1 gm of powdered root was accurately weighed and transferred in to a 500 ml conical flasks containing 100 ml water and boiled for 30 min, cooled and filtered into 100 ml volumetric flask and made the volume with water. The decoction was poured into ten stoppered test tubes in consecutive part of 1 ml; 2 ml; etc up to ten ml and adjusted the volume of each test tube with water to 10 ml and shaken them in lengthwise motion for 15 sec. Allowed to stand for 15 min and measured the height of the foam. The results were assessed as follows:

If height of foam in every tube was less than 1 cm the foaming index was considered less than 100. If height of the froth was higher than that of 1cm in every tube the foaming index was over than 1000. In such case repetitions was done by using a new series of dilutions of decoction in order to obtain the result. If height of foam in any test tube was 1 cm, and volume of the crude plant material decoction in that tube (a) was used to determine the index.

$$\text{Formula used for calculation of foaming index} = \frac{1000}{a}$$

a = Volume of decoction that was used for preparing the dilution in tube where foaming height was 1cm measured.

2.3.11 Determination of Tannins

Tannins are complex chemical compounds. They are occurring as mixture of polyphenols that are difficult to isolation and crystallize. They are capable of turning animal hide into pelt by binding proteins to form aqueous insoluble substances that are highly defiant to proteolytic enzyme. Powdered root (2 gm of each root) was

accurately weighed and placed into conical flask. Added 150 ml of distilled water and heated over boiling water for 30 min, cooled, transferred the mixture to 250 ml volumetric flask and diluted to volume with water. Allowed the solid material to settle down and filtered the liquid through filter paper, discarded the first 50 ml of filtrate. Evaporated 50 ml of extracts of root, to dryness, dried the residue in an oven at 105 °C for 4 h and weighed (T1). Took 80 ml of root extract, added 2 g of hide powder and shaken for 1 h. Filtered and evaporated 50 ml of clear filtrate to dryness. Dried the residues in an oven at 105 °C and weighed (T2). This is the amount of plant material that does not bind to hide powder. 2 g of hide powder was dispersed in 80 ml of water and shaken well for 1 hr. Filtered and evaporated 50 ml of clear filtrate to dryness. Dried the residues in an oven at 105 °C and weighed (T0).

Formula used for calculation of tannins percentage: = $\frac{[T1-(T2-T0)] \times 500}{W}$

Where W = the weight of the crude plant material 2 gm (Anonymous, 1996).

2.3.12 Determination of Bitterness Value

Medicinal plant materials have a strong bitter taste and act as appetizing agents. The bitter properties of plant materials are calculated by comparing the highest bitter amount of a plant extract of the materials with that of a quinine hydrochloride dilute solution.

2.3.12.1 Stock and diluted quinine sulphate solutions

Accurately weighed 0.1 g quinine hydrochloride (R) was dissolved in safe drinking water to produce 100 ml. 5 ml of this solution was further diluted up to 500 ml by means of safe consumable water. This quinine hydrochloride (S_q) stock solution of contained 0.01 mg/ml. Nine serial dilutions were made each containing 0.042, 0.044, 0.046, 0.048, 0.050, 0.052, 0.054, 0.056 and 0.058 ml solution of S_q and volume made up to 10 ml with safe drinking water and obtaining a concentration of 0.1, 0.2, 0.3 upto 1 milli gram/ml.

2.3.12.2 Plant Materials Stock and diluted solutions The stock solution was prepared of the concentration of 10 mg/ml in distilled water (S_T). 10 test tubes were used for serial dilution with 1 ml, 2 ml, 3 ml to 10 ml of (S_T) and final volume made up with safe drinking water to 10 ml.

2.3.12.3 Method

First of all washing the mouth with safe drinking water, 10 ml of the most weaken arrangement was tasted while twirling it in the mouth fundamentally close to the base of tongue 30 sec. After 30 sec the solution was spit out and it was ascertained for 1 min whether a delayed sensation for bitterness existed. Then mouth was rinsed water and after that highest concentration was not tasted until at least 10 min. The lowest concentration at which material continues to rouse a bitter feeling after 30 sec was referred the threshold bitter concentration. After a first series of test, rinsed the mouth systematically wash with safe consumption of water until no bitter feeling remains, wait at least 10 min before carrying out second test.

$$\text{Formula used for bitterness calculation} = \frac{2000 \times C}{A \times B}$$

Where A = Concentration of stock solution (S_q) mg/ml, B = Volume of (S_T) milli litre tube with bitter concentration, C = amount of quinine hydrochloride (in mg) tube with threshold bitter concentration (Anonymous, 1996).

2.3.13 Determination of Haemolytic Activity

Haemolytic activity of plant material is carried out for detection of saponins. It is determined by comparison of plant material extract and reference material saponin which has activity of 1000 unit per g. The erythrocyte suspension was prepared by one tenth of its volume with sodium citrate (36.5 g/L) filling in a glass stoppered flask. Sufficient volume of blood freshly collected from healthy rat was introduced to it and shaken immediately. 1 ml of citrated blood was further diluted with 50 ml phosphate buffer of pH 7.4. Reference solution was freshly prepared by dissolving 10 mg glycyrrhetic acid, (Himedia) in phosphate buffer pH 7.4 to make 100 ml.

2.3.13.1 Preliminary test

The alcoholic and aqueous extract (1 g) of root 0.1 ml, 0.2 ml, 0.5 ml and 1ml were taken and adjusted the volume in each tube with phosphate buffer to 1 ml. In each tube 1 ml of 2% blood suspension was added. Gently inverted to mix the tubes, to avoid the formation of foam. Tubes were shaken after 30 min interval. Then allowed to stand for 6 h at room temperature. Examined the tubes and recorded the dilution at which total haemolysis had occurred, as indicated by clear, red solution. The alcoholic extract of root has shown haemolytic activity in highest concentration i.e. 1 ml. Therefore further dilutions were done as follows.

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A serial dilution of alcoholic extract of root was prepared by using 13 test tubes in a concentration of 0.40, 0.45, and 0.50 up to 1 mg/ml and adjusted the volume in each tube with phosphate buffer to 1 ml. 1 ml of 2% blood suspension was added in each tube. Tubes were observed for haemolysis after 24 h. A serial dilution of glycyrrhizinic acid was prepared in the same manner. Calculated the quantity of glycyrrhizinic acid (g) that produces total haemolysis.

The haemolytic potential of the plant material calculated using the following formula :

$$\text{Haemolytic activity} = \frac{1000 \times a}{b}$$

1000 = the haemolytic activity of saponin (R) which is defined).

a = amount of saponin (R) that produce total haemolysis.

b = amount of plant extract that produce total haemolysis (g) (Anonymous, 1996).

2.3.14 Determination of Microbial Count (Anonymous, 1996)

2.3.14.1 Total viable aerobic bacterial count

Culture media

Soybean casein digest agar medium

Ingredients	Quantity (g)
Pancreatic digest of casein	=15 gm
Papaic digest of soybean meal	=05gm
Sodium chloride	=05gm
Agar	=15gm
Water	q.s. 1000 ml

Mixed all the contents and sterilized it by autoclaving at 121°C. Adjusted the pH to 7.3 ± 0.2.

Soybean casein digest medium

Ingredients	Quantity (g)
Pancreatic digest of casein	=17gm
Papaic digest of soybean meal	=03gm
Sodium chloride	=05gm
Dibasic potassium phosphate	=2.5gm
Dextrose	=2.5gm
Water	q.s. 1000 ml

All The ingredients were dissolved in distilled water and warmed slightly. After cooling it to room temperature, the pH was adjusted to 7.1 ± 0.2. It was sterilized by autoclaving at 121°C for 30 minutes.

Method

Each Sample (10ml) in separate was transferred to 100 ml of SCDM and mixed well in an incubator shaker at 125 rpm for 1-4 hours, for revivification of microorganisms.

1 ml of sample was pipetted out from SCDM broth medium into pre-sterilized petri-

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plates (180°C for 2 hours) and 15-20 ml of soybean casein SCDAM was added. The contents were mixed properly for uniform distribution and the SCDAM plates were incubated in a bacteriological incubator at 35°C for 48-96 h. After incubation total number of bacterial colonies was counted using colony counter and CFU / ml was calculated using the following formula:

$$\text{CFU / ml} = \frac{\text{Total counted colony on agar plates} \times \text{dilution}}{\text{Initial sample weight taken}}$$

2.3.14.2 Determination for *E. coli*

Culture media

Macconkey agar medium

Ingredients	Quantity (g)
Pancreatic digest of gelatin	= 17gm
Peptone	= 3gm
Lactose	= 10gm
Sodium chloride	= 5gm
Bile salts	= 1.5gm
Agar	= 13.5gm
Neutral red	= 30gm
Crystal violet	= 1.0gm
Water to	q.s. 1000ml

The ingredients were boiled in water for 1 minute to affect solution and after adjustment of pH to 7.1 ± 0.2, sterilization was done.

Macconkey broth medium

Ingredients	Quantity (g)
Pancreatic digest of gelatin	= 20gm
Lactose	= 10gm
Dehydrated ox bile	= 5gm
Bromocresol purple	= 10gm
Water	q.s 1000 ml

The adjustment of pH to 7.3 ± 0.2 was done and it was sterilized.

Method

Aseptically 10ml of sample was transferred to 100ml lactose broth/soyabean casein digest broth medium and the media was incubated at 37°C for 24 h. The flask was examined for growth and the contents were mixed by gentle shaking. 1 ml of the

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enriched culture was pipetted into the tubes containing 10ml MacConkey,s broth and incubated at 35°C for 26 h. Concomitantly, streaking on the surface Mac Conkey,s agar medium was done using a loopful of enriched culture and the plates were incubated at 37°C for 24 h.

2.3.14.3 Determination for *Salmonella typhi*

Culture media

Selenite F broth

Ingredients	Quantity (g)
Peptone	= 05gm
Lactose	= 02gm
Disodium hydrogen phosphate	= 10gm
Sodium hydrogen selenite	= 04gm
Water	q.s. 1000 ml

All ingredients were boiled in water for 1 min to affect solution and after adjustment of pH to 7.1 ± 0.2 , sterilization was done.

Method

1.0 ml of the enriched culture was added to the tubes containing 10ml of selenite F broth. The tubes were incubated at 35°C for 48 hours and observed for the presence of turbidity.

2.3.14.4 Total fungal count

Culture media

- (i) Soyabean casein digest agar medium
- (ii) Soyabean casein digest medium

Method

The culture media were prepared as above and 10ml of each sample was transferred to 100 ml of SCDM and mixed well for 1-4 in incubator shaker at 125 rpm for revivification of microorganism. 1 ml of sample was pipetted out from SCDM broth medium into pre-sterilized petri plates (180°C for 2 h) and 15-20 ml of SCDA was added. The contents were mixed properly for uniform distribution and the SCDA plates were incubated in BOD incubator at 25°C for 5-7 days. After incubation total number of fungal colonies was counted with the help of colony counter and CFU per ml was calculated using formula:

$$\text{CFU / ml} = \frac{\text{Total counted colony on agar plates} \times \text{dilution}}{\text{Initial sampleweight taken}}$$

2.3.14.5 Determination for *Pseudomonas aeruginosa*

Culture media

Cetrimide agar medium

Ingredients	Quantity (g)
Pancreatic digest of gelatin	= 20gm
Magnesium chloride	= 1.4gm
Potassium sulphate	= 10gm
Cetrimide	= 0.3gm
Agar	= 13.6gm
Glycerin	= 10gm
Water	q.s. 1000 ml

The ingredients were boiled in water for 1 minute to affect solution and after adjustment of pH to 7.1 ± 0.2 , sterilization was done.

Method: 1.0 ml of the culture was added to the plates containing cetrimide agar media, mixed and incubated at 35°C to 37°C for 24 to 48 hours and observations for microbial growth were made.

2.3.14.6 Determination for *Staphylococcus aureus*

Culture media

Vogel Johnson agar medium (VJA)

Ingredient	Quantity (g)
Pancreatic digest of casein	= 10gm
Yeast extract	= 5 gm
Mannitol	= 10gm
Dibasic potassium phosphate	= 5gm
Lithium chloride	= 5gm
Glycine	=10gm
Agar	16
Phenol red	25
Water	q.s. 1000 ml

All ingredients were made in to solution by heating and cooled to approximately 45°C & 20 ml of one precent w/v solution of potassium tellurite was added to it. The pH was adjusted to 7.4 ± 0.2 and the contents were sterilized.

Method

Enriched culture was streaked on the surface of VJA media and incubated at 35°C for 24 hours and observed for the presence of growth

2.3.15. Paper Partition Chromatography of Amino Acids (Das, 2005; Evans, 2006; Sharma and Ali, 1992).

Amino acids are the fundamental units of proteins. The proteins are found in every alive cell. The amino acids which can be synthesized by the living cells are called non-essential amino acids, while those which cannot be synthesized are called essential amino acids and must be supplied by diet. The essential amino acids are Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Valine. The Non-essential amino acids are Alanine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Proline, Tryptophan, Glutamine, Asparagine, Serine and Tyrosine etc.

Carbohydrates are the most abundant organic molecule in nature. These are the carbon compounds that contain large quantities of hydroxyl groups. The simplest carbohydrates also contain either an aldehyde moiety or a ketone moiety. All carbohydrates can be classified as either monosaccharides, oligosaccharides or polysaccharides e.g. Ribose, Ribulose, Xylulose Glucose, Galactose, Mannose, Fructose, Erythrose (Styler, 1997).

2.3.15.1 Preparation of extract

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 mother liquor obtained after removing the proteins was concentrated for detection of amino acids in Free State and carbohydrates

2.3.15.2 Paper chromatography

Chromatographic Whatman paper number one sheets (Qalligens) were used for paper chromatography. The starting line was marked two centimeter above from the base. To obtain the desired concentration of the extract on the paper, the spots were applied repeatedly at the same point. The spots were kept at a distance of two centimeter apart for the amino acid identification

The solvent systems used were n-Butanol: Acetic acid: Water (4:1:1) (BAW) for amino acids and *n*-butanol: glacial acetic acid: water (2:1:1) for carbohydrates.

The chamber was saturated in 16 h prior the experiment with respective solvent systems. Care was taken so as not to touch the paper with fingers. The papers were developed in descending manner. Air dried chromatograms were sprayed with 0.2% w/v solution of Ninhydrin in acetone for amino acids and aniline hydrogen pthalate for carbohydrates, heated at 110°C in oven. Pink to violet colour were visualized for amino acids and yellow to dark brown for carbohydrate.

R_f values for each spot was calculated

$$R_f = \frac{\text{Distance travelled by the solute from the start}}{\text{Distance travelled by the solvent from the start}}$$

2.3.16 Preliminary Phytochemical Screening (Rangari, 2000)

2.3.16.1 Preparation of the extract

Around 20 g of air dried powdered roots were extricated with ethanol in a soxhlet apparatus for 72 hrs and watery concentrate was arranged by maceration with refined water for 24 h to get the fluid concentrate. Concentrated ethanol and watery concentrate in revolving vaccum evaporator and unrefined ethanol concentrate was fractioned viz. petroleum ether, chloroform, ethyl acetic acid, ethanol and aqueous. The concentrates were screened for the presence of different phytoconstituents.

2.3.16.2 Test for alkaloids

A little portion of the dissolvable free petroleum ether, chloroform, ethyl acetic acid derivation, ethanol and water extricates independently with a couple of drops of dilute hydrochloric acid and filter. The filtrates were tried with different alkaloidal reagents, for example, Mayer's reagent (cream encourage), Wagner reagent (reddish brown precipitate)and Dragendorff's reagent (orange brown precipitate).

Mayer's reagent: Mayer's reagent few drops of were included every concentrate and watched development of the white or cream hued precipitates.

Dragendorff's reagent: Dragendorff's reagent few drops of were included every concentrate and evelopment of the orange yellow or brown hued ppt.

Wagner reagent: Wagner reagent Few drops of were included every concentrate and watched development of the red colored ppt.

2.3.16.3 Test for carbohydrates: Dissolve little amounts of alcoholic and watery extracts, independently in 4 ml of refined water and filter. The filtrate may be subjected to different tests to detect the existence of carbohydrates.

Molisch's Test: To around 2 ml of concentrate couple of drops of α -naphthol (20% in ethanol) were included. At that point around 1 ml of concentrated sulphuric acid was included at the edge of the tube. In the event that red violet ring showed up at the intersection of two layers. It showed the vicinity of carbohydrates.

Fehlings Test: 1ml of copper sulphate in alkaline conditions (Fehling's reagent) was included to the filtrate of the root separate in refined water and warmed in a steam shower. Brick red precipitates showed up if sugars are present.

2.3.16.4 Test for glycosides

Hydrolysed another little part of the concentrate with weaken hydrochloric acid for couple of hrs in water bath and subjected the hydrolysate with borntrager's, and liebermann-burchard's, keller-killani tests to recognize the presence of glycosides.

Keller-Killani Test: 1ml of glacial acetic acid containing hints of FeCl_3 and 1 ml of concentrated H_2SO_4 was added to the concentrate deliberately. Appearance of shading showed up which affirmed the vicinity of glycosides in the root extricates.

Borntrager's test: 1ml of benzene and 0.5 ml of dilute alkali solutions were poured in the extract. dark brown shading which shows the vicinity of glycosides in the extract.

2.3.16.5 Test for phenolic compound and tannins

Taken little amounts of different concentrates independently in water and test for the vicinity of phenolic mixes and tannins with weaken ferric chloride arrangement (5%) and lead acetic acid test.

Ferric chloride test: Added ferric chloride solution (5%), if green/ blue colour was seen in all the segments it was because of the vicinity of phenolic components. Colour showed up which demonstrate the vicinity of phenolic compound.

Lead acetic acid vation test: Added few drops of lead acetic acid solution (5%) were added to the different extract of the root. The appearance of white ppt affirm the vicinity of phenolic components.

2.3.16.6 Test for flavonoids

Ammonia test: Filter paper strips were dunked in the different extracts and ammoniated. The filter paper transformed its colour to yellow which demonstrates the vicinity of flavonoids.

Pew test for flavonoids: To 1ml of the every concentrates, a bit of metallic magnesium/zinc was included trailed by expansion of 2 drops of concentrated

hydrochloric acids. An brown shading the vicinity of flavonoids in the different extracts.

2.3.16.7 Test for proteins and free amino acids

Added a couple of drops of various extracts in a couple ml of refined water and subjected to ninhydrin and million's and biuret tests .

Millon's test: To 2 ml of extract filtrate, few drops of million's reagent was added. A red colour affirms the vicinity of proteins and free amino acids.

Biuret test: In the ammoniated filtrate few drops of 0.02% copper sulphate solution was included. A red colour confirms the vicinity of free amino acids and proteins.

Ninhydrin test: To each of the extract filtrate, lead acetate solution was included to accelerate tannins and filtered. The Filtrate was spotted on a paper chromatogram, splashed with ninhydrin reagent and dried at 110o C for 5 minutes. Violet spots if seen affirmed the vicinity of proteins and free amino acids.

2.3.16.8 Test for saponin

Foam test: : Dilute 1 ml of alcoholic and fluid concentrates independently with refined water to 20 ml and shake in a graduated barrel for 15 minutes. An one centimeter layer of froth shows the vicinity of saponin.

Sodium bicarbonate test: To the couple of milligrams of concentrate couple of drops of sodium bicarbonate were included and shaken well. Arrangement of honeycomb like foaming shows positive test for saponins.

2.3.16.9 Test for phytosterol and triterpenes

Liebermann-Burchard's test: The hydro-alcoholic concentrate was shaken with chloroform and couple of drops of acidic anhydride were included chloroform extricate alongside a couple of drops of concentrated sulphuric corrosive from the side of the tube. The presence of blue to block red shading shows the vicinity of sterol and triterpenes.

Hesse's response: The deposit was broken down in chloroform (4 ml) and equivalent amount of concentrated sulphuric corrosive was then at the edge of the tube. The arrangement of the pink hued ring, which is on shaking diffused in both the layers, demonstrated the vicinity of sterols in the concentrate.

2.4 Column chromatography: Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or

the support coated with a liquid stationary phase may fill the whole side volume of the tube (packed column) corning glass column was used for the present study.

Extraction: The dried powdered aerial root of *Ficus lacor* (2.5 kg) was subjected to hot continuous extraction with ethanol using soxhlet apparatus for 72 h till solvent became colourless. The liquid was concentrated in rotary vaccum evaporator. A thick dark brownish viscous mass (100 g) was obtained.

The dried powdered root of *Murraya koenigii* (2 kg) was subjected to hot continuous extraction with ethanol using soxhlet apparatus for 72 h till solvent became colourless. The liquid was concentrated in rotary vaccum evaporator. A thick dark brownish viscous mass (85 g) was obtained

Packing of the column: Silica gel for column (100-120 mesh size)

Mobile phase: Solvent system employed for column in increment of polarity were pure petroleum ether, petroleum ether: chloroform (19:1), (9:1), (3:1), (1:1), pure chloroform, chloroform : methanol 19:1), (9:1), (3:1), (1:1), methanol.

2.5 Pharmacological screening (Ghose, 1984; Kulkarni, 1999)

The various extracts and fractions were subjected to pharmacological screening to identify fractions responsible for anti-inflammatory and anti-oxidant activities. Screening was done by using various models to identify the mechanism of action.

2.5.1 Determination of LD₅₀ value and acute toxicity: (Kulkarni, 1999)

Rats in groups of 6 were administered intraperitonealy with different doses of the fractions from the three drugs by the staircase method, starting from 10 mg/kg and increasing dose by a factor 1.5 if there was no mortality and decreasing subsequent dose by a factor .7 in case there was mortality (Ghosh 1984). Least tolerated (100 % mortality) and most tolerated (0 % mortality) were determined by hit and trial method for various extracts and fractions. Corrections for 0% and 100% mortality were done by the formulas:

100 (0.25/n); For 0% mortality: and, 100 ($n - 0.25 / \sqrt{n}$); For 100% mortality.

S.E. of LD₅₀ = Log Dose with highest mortality- Log Dose with lowest mortality/ n
Doses were selected between these two and any mortality observed for 24 hrs and the number of deaths noted. A curve of log dose versus probit value was plotted to get dose for probit value 5 which was taken to be LD₅₀. Dose range well below LD₅₀ was selected for study.

Dose planning and Grouping of animals:

Doses equivalent of or less than 1/10th of the doses corresponding to LD₅₀ values and producing no gross behavioural changes or cvs changes were used. Fractions showing

no CNS or CVS effects even at higher doses were used in wider dose ranges as compared to fractions which showed some changes at higher doses. For the later fractions a narrower dose range (10, 20, 40, 60, 80 and 100mg/kg) was used for study of the dose response relation for different parameters. For other fractions 10, 20, 50, 100, 120 and 150 mg/kg was the dose gradient used to study the dose response relationships.

Grouping:

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

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

2.5.2 Screening for anti-inflammatory and anti-arthritis Activity

2.5.2.1 Inhibition of Carrageenan induced, paw edema in rats (Winter et al 1962):

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

1. Normal saline treated control
2. Untreated diseased animals
3. Reference group treated with indomethacin before carrageenan
4. 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 affection 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 2.5 mg/kg 1hr preceding the carrageenan infusion. Rate restraint of edema in respect to a control gathering was ascertained as depicted by Winter *et al.*

2.5.2.2 Inhibition of histamine and serotonin induced paw edema in rats (Singh et al 1996).

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

1. Vehicle treated normal control
2. Untreated diseased animals
3. Reference group treated with indomethacin before carrageenan
4. 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.

2.5.2.3 Inhibition of formalin induced paw edema in rats (Hosseinzadeh and Younesi 2002)

Animals were separated into groups of six each group. Acute inflammation was actuated by subaponeurotic infusion of 0.1 ml of 2% formalin one hour after i.p. organization of different doses of extracts or fractions, Diclofenac (5 mg/kg), or just vehicle (solution of 2.5% DMSO and 2.5% tween 20) in individual gatherings. The volume of paw was resolved one, two, and four hours taking after the infusion of formalin by plethysmometer. For ceaseless aggravation mull over, the above creatures were further treated with the parts, Diclofenac or vehicle, once day by day, for 9 sequential days and a second infusion of formalin was given on the third day. The every day changes in the volume of paw were measured plethysmographically.

2.5.2.4 Inhibition of Adjuvant induced arthritis in rats (Newbould 1963)

Animals were divided into groups of 6 each group. Creatures were separated into gatherings of 6 each as prior. Joint inflammation was affected by intradermal infusion of 0.5 ml of a 5 mg/ ml suspension of warmth executed *Mycobacterium tuberculosis* in fluid paraffin into the plantar surface of the rear paws. The clinical highlights of adjuvant actuated joint pain (AIA) showed as erythema, induration and edema, and displayed in various joints as takes after: (an) onset: clinical signs around days 8–10; (b) early stage: dynamic seriousness of the clinical signs through the following 7–10 days; and (c) late stage: unconstrained relapse amid the following 10–14 days.

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Every one of the four paws were inspected and evaluated for seriousness and loci of the joint injuries (erythema, swelling and induration) which grew on the paws by the 15th day and surveyed on a subjective 5-point size of ligament score (0-4). Rats were evaluated day by day for indications of joint inflammation upto 28th day post-CFA.

The maximal ligament score every rodent was situated at 16 (greatest of 4 points \times 4 paws), where;

0 = no indications of illness;

1 = signs including the lower leg/wrist;

2 = signs including the lower leg in addition to tarsals (proximal piece of the rear paw) and/or wrist in addition to carpals of the forepaw;

3 = signs reaching out to the metatarsals or metacarpals;

Furthermore, 4 = extreme signs including the whole rear or fore paw.

Animals with affirmed joint pain were differentiated into group of 6, with comparable mean ligament scores and infused with various fractions, at different doses of fractions or reference drug (indomethacin 2.5 mg/kg) intraperitonealy in two dosages (one measurement relating to ED50 dosage for portions as decided for hindrance of carrageenan induced inflammation, (or 20 mg/kg) and a higher dosage well beneath the poisonous measurement, which was 100-150 mg/kg for different parts) day by day for next 7 days from 15th day onwards. Mean joint scores got for every after a long time for treated gatherings were contrasted and fitting scores of the control group.

The progressions in body weight were recorded on 7th, 14th and 21st day and toward the end of 28th day. Rats were yielded on the 29th day by beheading. The blood was gathered and biochemical parameter like hemoglobin substance, RBC, WBC and ESR measured. Plasma was differentiated from the blood gathered with EDTA. IL-1 and TNF- α measured. Promptly in the wake of yielding, liver, kidney and spleen were divided, organ weight changes on 28th day & homogenized enzyme estimation.

2.5.2.5 Lysosomal enzyme inhibitory activity (Geeta and Varalaxmi 1999)

It was performed along with determination of adjuvant arthritis inhibition studies. As described, rats were divided into groups of 6. Reference control group was given Indomethacin (2.5 mg/kg)

Enzyme assays: The activities of lysosomal enzymes were investigated in liver, plasma, kidney and spleen.

Acid phosphatase: It was measured by the method of King (1965), on the basis of the action of the enzyme on disodium phenyl phosphate substrate mixture containing 1.5ml 0.01 M substrate, 1.5ml 0.1M citrate buffer pH 4.8 and 0.5ml tissue homogenates) to liberate phenol after incubation at 37°C for 30 mins. The reaction was then arrested by addition of 1ml 10% Trichloro acetic acid and the released phenol treated with 1 ml 15% Sod. Bicarbonate and Folin's Phenol reagent, incubated at 37°C for 10mins and colour developed measured at 640 nm and compared to standard phenol solutions and blank treated in similar fashion. The activity of acid phosphatase was expressed as μ mol. of Phenol liberated /min/mg protein at 37°C.

2.5.2.6 Screening for Antioxidant activity:

Plants are a usual source of pharmacologically active chemical compounds known as phytoconstituents (Farnsworth, 1994). Phytoconstituents found to go about as cell by searching free radicals, and numerous have helpful potential with the expectation of complimentary radical related ailments. Responsive oxygen species (ROS) including singlet oxygen, hydroxyl radicals, superoxide radicals and hydrogen peroxide are often produced as by results of natural response (Kikuzaki and Nakatani 1993). In any case, these ROS created by daylight, UV light, ionizing radiation and metabolic process of action have a wide range of obsessive impacts, for example, carcinogenesis, Arthritis, DNA harm and different degenerative ailments, for example, neuro-degenerative disease, maturing and cardiovascular diseases (Osawa, 1994; Noda *et al.* 1997).

2.5.2.6.1 Total phenolic content

The total phenolic substance in the concentrate was calculated using the Folin-Ciocalteu's reagent (FCR) as indicated by Molan *et al.* 2009. Every sample (0.5 ml) was blended with 2.5 ml of FCR (diluted 1:10, v/v), and 2 ml of Na₂CO₃ (7.5%, w/v) was included. The absorbance was then measured at 765 nm after brooding at 30°C for 90 minutes. Results were communicated as gallic corrosive equivalents (mg of gallic acid/g of dried extract).

2.5.2.6.2 Total flavonoid content

Total flavonoid substance of ethanol concentrate was calculated utilizing a colorimetric process (Zhishen *et al.*, 1999). Quickly, every specimen (0.5 ml) was blended with 2 ml of refined water and after this 0.15 ml of a NaNO₂ solution 15%, w/v. Following 6 minutes, 0.15 ml of an AlCl₃ solution 10% w/v was added and

permitted to remain for 6 minutes, then 2 ml of NaOH solution 4%, w/v was added to the mixture. Momentarily, water was added to convey the last volume to 5 ml, and after that the mixture was precisely blended and permitted to remain for an additional 15 minutes. Absorbance of the solution was calculated on 510 nm versus arranged blank water. Results were shown as catechin equivalent (mg of catechin/gm of dried concentrate).

2.5.2.6.3 Free radical scavenging activity

Scavenging potency of diphenyl- 2-picrylhydrazyl (DPPH) radicals of ethanol extract or catechin was preformed according to the method reported by Molan *et al.*, 2009, with minor modifications. Assays process was performed in 3 ml of reaction mixtures composed of 2.0 ml of 0.1 mM DPPH ethanol solution, 0.9 ml of 50 mM Tris-HCl buffer (pH 7.4) and 0.1 ml deionized water (as control) or test plant concentrate. Following 30 minutes of standing at room temperature, absorbances of the reaction mixtures at 517 nm were noted. The inhibitory impact of DPPH was computed by following formula:

$$\text{Percentage inhibition} = [(\text{Absorbance control} - \text{Absorbance sample}) / \text{Absorbance control}] \times 100$$

2.5.2.6.4 NO scavenging activity

The scavenging impact of ethanol concentrate on nitric oxide was measured according to Marcocci *et al.*, 1994. Sodium nitroprusside (5 mM) in phosphate-buffered saline pH 7.4 was blended with diverse concentrations of the test sample (100, 200 -1000 mg/ml) and incubated at 25 °C for 150 minutes. After incubation, , nitrite delivered from sodium nitroprusside was measured by Griess reagent, one percent sulfanilamide solution in 5 percent phosphoric acid and 0.1 percent 1-naphthylethylenediamine dihydrochloride in H₂O. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and consequent coupling with 1-naphthylethylenediamine dihydrochloride was noted at 570 nm. Catechin used as a positive control. The percentage of NO scavenging was calculated using following formula:

$$\text{Percentage inhibition} = [(\text{Absorbance control} - \text{Absorbance sample}) / \text{Absorbance control}] \times 100$$

2.5.2.7 Histological analysis

All preparations were stored in six percent formaldehyde for 24 hours. Decalcification in ethylenediamine tetra acetic acid was subsequently done and the planning were set in paraffin.then after the exclusion of paraffin, 5-μm thick section were cutted.

MATERIALS AND METHODS

For the measurement of the histological arthritis scores, the joints sections were stain with haematoxylin and eosin. All cases of diseased and other, three sections per knee joint were evaluated using Almicro 50Hz 200AC Microscope with camera at 10 X 0.25 X magnification and scored using a semi quantitative scale.

The extent of acute joint inflammation: was defined by the quantity of infiltration of the synovial membrane by polymorph nuclear leucocytes, the exudation of granulocytes cells in the joint space, the presence or absence of fibrin exudation in the joint space and periarticular inflammation. Each parameter was scored using a 0-3 scale, where:

0 = there was no changes, 1= mild affect; 2 = moderate changes, and 3 = severe change.

A maximum total score of 12 was therefore possible for different groups for acute Inflammation.

Chronic joint inflammation was evaluated based on the hyperplasia of synovial lining cells, infiltration by mononuclear cells and fibrosis of synovial membrane or periarticular tissue. These were evaluated with a score of 0-3, resulting in a maximum total score of 9 in a particular group.

The degree of the injures to articular cartilage and adjoining bone structures was evaluated on the basis of cell necrosis, structural bone defects and cartilage defects and evaluated on a scale of 0-4, where,

Score 0=no damage, 1= <5% of the cartilage surface affected, 2 = 5 -10% of the cartilage surface influenced, 3 = 10-50% of the ligament surface influenced, 4= > 50% of the ligament surface influenced (maximal aggregate score of 4).

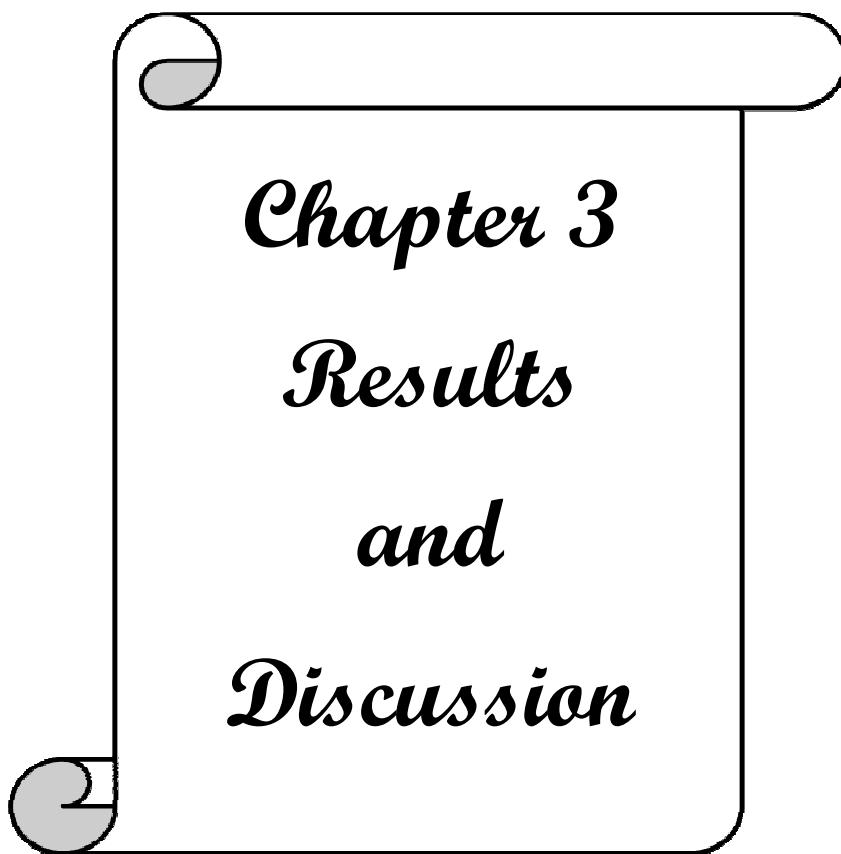
Safranin O recoloring was performed to gauge the proteoglycan content in the ligament. Keeping in mind the end goal to acquire similar histological result all slides were recolored utilizing precisely the same system.

First and foremost, the recoloring power (red) at the epiphyseal development plate of the femoral condyle of non-joint and ligament creatures was measured. The number juggling mean got from these qualities was utilized as a kind of perspective esteem (100%). The estimations of articular ligament occurred at the most distal purpose of the arch of the femoral condyle. For every situation, qualities were gotten for the shallow layer, center layer, and profound layer of the hyaline ligament, and in addition for the calcified ligament layer. Information was communicated as a rate of the reference esteem. Accordingly, the estimations of the contra parallel, non-

ligament knee joint (left) were subtracted from the ligament knee (right), bringing about negative values on account of proteoglycan loss.

2.5.2.8 Statistical analysis

The data acquired from animal trials were communicated as mean standard error (\pm S.E.M.). Statistical differences between the treatment and the control were assessed by ANOVA and Students–Newman–Keuls post hoc tests. Significance of data was expressed as $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$.



Chapter 3

Results

and

Discussion

3.1 PHARMACOGNOSTIC STUDIES

3.1.1. 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 root breaks with a short fracture when dry. The odour was characteristic. The mature aerial roots is 11-19 mm thick, grey, light green, slightly spiral, thickness varies with the age of the tree. Surface is rough due to the presence of longitudinal and transverse row of lenticels, circular and prominent, fracture short and fibrous (Figure 3.1).

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.



Figure 3.1: Morphology of *Ficus lacor* aerial roots



Figure 3.2: Morphology of *Murraya koenigii* roots

RESULTS AND DISCUSSION

3.1.2 Microscopic Study

Anatomical investigations of root showing the following features:

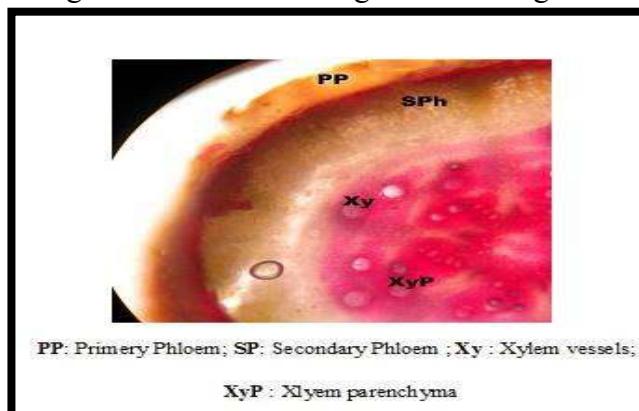


Figure 3.3: Transverse Section of Aerial roots of *Ficus Lacor*

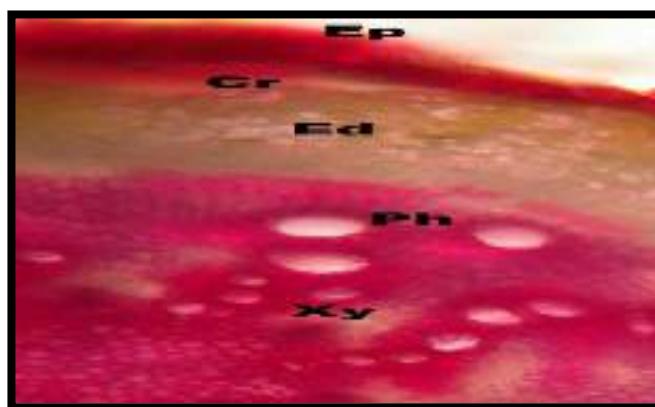


Figure 3.4: Transverse Section of *Murraya koenigii* roots

3.1.3 Powder Studies

3.1.3.1 Microscopical study

The root powder was studied under microscope shows the presence of

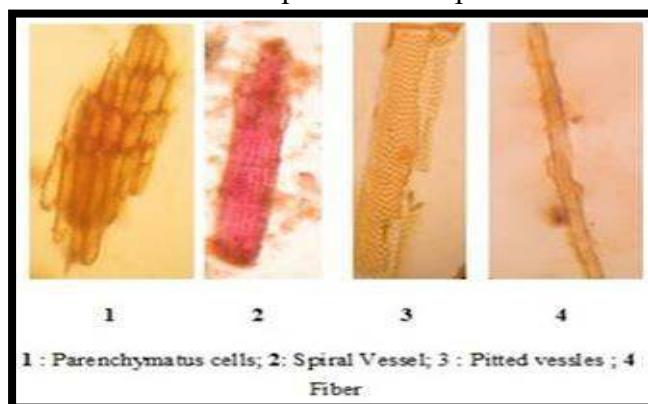


Figure 3.5: Powder Microscopy of *Ficus lacor* aerial roots

RESULTS AND DISCUSSION

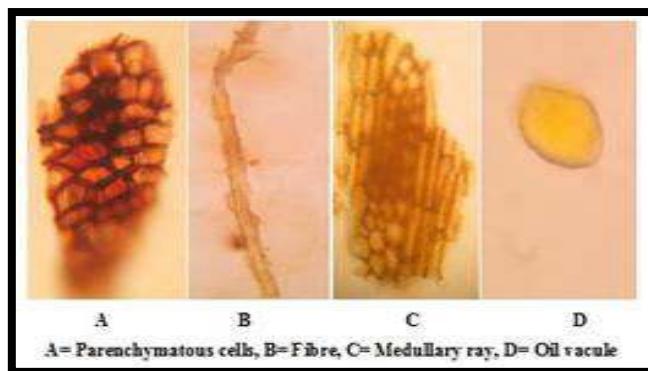


Figure 3.6: Powder Microscopy of *Murraya koenigii* roots

3.1.4 Histochemical Colour Reactions

Table 3.1: Behaviour of transverse section with different chemical reagents

Sr. No.	Reagents	Test for	Nature of change in histochemical zone	Degree of change (F. lacor)	Degree of change(M. koenigii)
1.	Phloroglucinol+HC 1	Lignin	Xylem vessels become pink	+	++
2.	Millon's reagent	Proteins	Yellow colour	+	+
3.	Iodine solution followed by H_2SO_4	Cellulose	Cellulose wall become violet	+	+
4.	Dragendroff's reagent	Alkaloid	Brown colour	-	++
5.	Wagner's reagent	Alkaloid	Dark yellow colour	-	++
6.	H_2SO_4 solution	Sterol	Red colour	+	++
7.	Libberman - Burchard reagent	Terpenes	Pink colour	+	+
8.	Acetic anhydride and H_2SO_4 solution	Sterol	Black colour	++	-
9.	$FeCl_3$ solution	Tannins	Dark green to black colour	-	-
10.	Iodine solution	Starch	Light bluish	+	+
11.	Caustic alkali+HCl	Calcium oxalate	No change	-	-
12.	Aqueous. KOH solution 10%+ H_2SO_4	Suberin	Light brown	-	-
13.	Chloroform+ H_2SO_4	Sterol	Red colour	+	+

++ High; + Moderate; - Absent

RESULTS AND DISCUSSION

3.1.5 Histochemical Colour Reactions Powder Studies

Table 3.2: Roosot powders behaviour with different chemical reagents

Sr. No.	Treatment	Colour of <i>Ficus lacor</i> aerial roots powder	Colour of <i>Murraya koenigii</i> roots powder
1	Powder as such	Brown	Light Yellow
2	Powder + 1 N HCl	Dark brown	Dark brown
3	Powder + 1N NaOH	Light brown	Light brown
4	Powder + Acetic Acid	Light pinkish	Reddish brown
5	Powder + 5% Ferric chloride	Cream	Light Cream
6	Powder + Picric acid	Greenish yellow	Fluorescent light yellow
7	Powder + HNO ₃ + Ammonia solution	Brown	Light Brown
8	Powder + 5% Iodine	Black brown	Dark brown
9	Powder + 1N HNO ₃	Brown green	Brown

3.1.6 Fluorescence nature of *Ficus lacor* aerial roots powder under ultra violet) and visible radiations

Table 3.3 A: Fluorescence nature of *Ficus lacor* aerial roots powder under ultra violet (UV) and visible radiations

Sr. no.	Treatment	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Powder as such	Parrot brown	Light green	Light brownish
2	Powder + 1N HCl	Dark green	Light brown	Light brown
3	Powder + 1N NaOH	Light brown	Light brown	brown
4	Powder + 50% HNO ₃	Dark brown	Light brown	Brown
5	Powder + Acetic acid	Yellowish	Light yellow	Light brown
6	Powder + Picric acid	Light brown	Dark brown	Light brown
7	Powder + 1N NaOH in methanol	Light greenish	Dark brown	Light brown
8	Powder + FeCl ₃	Creamish	Light brown	Light brown
9	Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Light yellowish	Light greenish	Light brown
10	Powder + 1N HCl + Nitrocellulose in amyl acetate	Light greenish	Brown	Light brown
11	Powder + 1N NaOH + Nitrocellulose in amyl acetate	Light greenish	Dark green	Light brown

RESULTS AND DISCUSSION

Table 3.3B: Fluorescence nature of *Murraya koenigii* root powder under visible and ultra violet (UV) radiations

Sr. no.	Treatment	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Powder as such	Fluorescent yellow	Light brown	Light Yellow
2	Powder + 1N HCl	Dark green	Light brown	Light brown
3	Powder + 1N NaOH	Dark brown	Light brown	Reddish brown
4	Powder + 50% HNO ₃	Dark brown	Light brown	Brown
5	Powder + Acetic acid	Yellowish	Light yellow	Light brown
6	Powder + Picric acid	Fluorescent yellow	Dark brown	Light yellow
7	Powder + 1N NaOH in methanol	Light greenish	Dark brown	Light brown
8	Powder + FeCl ₃	Cream brown	Light brown	Light brown
9	Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Light yellowish	Light greenish	Light brown
10	Powder + 1N HCl + Nitrocellulose in amyl acetate	Light brown	Brown	Brown
11	Powder + 1N NaOH + Nitrocellulose in amyl acetate	Light greenish	Light brown	Light brown

3.1.7 Total ash, water soluble ash, acid insoluble ash and sulphated ash of roots powder

Table 3.4: Total ash, water soluble ash, acid insoluble ash and sulphated ash of roots powder

Sr. No.	Ash	Percentage in FL	Percentage in MK
1	Total ash	14.15	11.25
2	Water soluble ash	8.57	10.57
3	Acid insoluble ash	10.75	8.75
4	Sulphated ash	6.00	7.02

RESULTS AND DISCUSSION

3.1.8 Extractive values

Table 3.5: 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

3.1.9 Crude fiber content, Loss on drying, Swelling index, Foaming index, Tanins contents, Bitterness value, Haemolytic value

Table 3.6: Crude fiber content, Loss on drying, Swelling index, Foaming index, Tanins contents, Bitterness value, Haemolytic value.

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 %

3.1.10 Microbial determination

Table 3.7 A: Microbial determination in various extracts of *Ficus lacor*

Extracts	Total bacterial count	Total fungal count	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Pet. Ether Extract	-	-	-	-	-
Ethyl acetate Extract	-	-	-	+	+
Chloroform Extract	-	-	-	-	-
Ethanol extract	-	-	-	-	-
Aqueous. Extract	-	+	-	-	-

+ + High; + Moderate; - Absent.

RESULTS AND DISCUSSION

Table 3.7 B: Microbial determination in various extracts of *Murraya koenigii*

Extract	Total bacterial count	Total fungal count	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Pet. Ether Extract	-	-	-	-	-
Ethyl acetate Extract	-	-	-	-	-
Chloroform Extract	-	-	-	-	-
Ethanol extract	-	-	-	-	-
Aqueous Extract	+	-	-	-	-

+ + High; + Moderate; - Absent.

3.1.11 Paper chromatography

Table 3.8: Amino acids analyzed by paper chromatography

Sr. No.	Amino Acids	R _f Value	Amino acids detected FL	Amino acids detected MK
1.	Alanine	0.32	+	-
2.	2-Aminobutyric acid	0.70	-	-
3.	Arginine	0.15	-	-
4.	Aspartic acid	0.16	-	-
5.	Glutamic Acid	0.18	-	-
6.	Glycine	0.16	-	+
7.	Histidine	0.14	-	-
8.	Lysine	0.18	-	-
9.	Methionine	0.37	+	-
10.	Norleucine	0.10	-	-
11.	Ornithine	0.10	+	-
12.	Phenylalanine	0.25	-	+
13.	Tyrosine	0.47	+	+

+ve : Detected; -ve : Not detected

Solvent system : n-butanol : Acetic acid : Water (BAW) (4:1:1, upper phase).

Spray reagent : Ninhydrin(0.5% in acetone/ ethanol)

RESULTS AND DISCUSSION

Table 3.9: Carbohydrates analyzed by paper chromatography

Sr. No.	Carbohydrates	R _f Value	Carbohydrate Detected in FL	Carbohydrate Detected in MK
1.	Galactose	0.646	+	+
2.	Maltose	0.60	-	-
3.	Lactose	0.622	+	-
4.	D-Ribose	0.525	-	+
5.	Sucrose	0.629	+	-
6.	Fructose	0.666	-	+
7.	D-Xylose	0.711	-	-
8.	L-Arabinose	0.666	-	-

+ve : Detected; -ve : absent

Solvent system : n-butanol : Acetic acid : Water (BAW)(4:2:1)

Detecting reagent : Anisaldehyde in sulphuric acid

3.1.12 Preliminary phytochemical screening

Table 3.10A: Preliminary phytochemical screening of *Ficus lacor*

Sr. no.	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	ALKALOIDS					
	Mayer's reagent	-	-	-	+	-
	Dragendorff's reagent	-	+	+	+	-
2.	GLYCOSIDES					
	Killer-Killani test	+	-	-	+	-
	Sodium nitropruside test	-	-	-	+	-
	Borntrager test	+	-	-	+	-
3.	CARBOHYDRATES					
	Molisch's reagent	+	-	-	+	+
	Fehling solution	+	-	-	+	+

RESULTS AND DISCUSSION

Sr. no.	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chloroform extract	Ethanol extract	Aqueous extract
4.	STEROLS					
	Liebermann-Burchard's test	+	-	+	+	-
	Salkowski test	+	-	-	-	-
	Hesses reaction	-	-	+	+	-
	Hersch reaction	-	-		+	-
5.	SAPONINS					
	Foam test	-	-	+	+	-
	Sodium bicarbonate test	-	-	+	+	-
6.	PHENOLIC COMPOUNDS & TANNINS					
	Ferric chloride solution	+	+	-	+	-
	Lead acetate solution	+	+	-	+	-
7.	PROTEINS & AMINO ACIDS					
	Biuret test	-	+	-	+	-
	Millon's reagent	-	-	-	+	+
	Ninhydrin reagent	+	+	-	+	+
8.	FLAVONOIDS					
	Shinoda/Pew test	+	+	-	+	+
	Ammonia test	+	+	-	+	+

+ve: Present; -ve: Absent

Table 3.10B: Preliminary phytochemical screening of *Murraya koenigii*

Sr. No.	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chlorofor m extract	Ethanol extract	Aqueous extract
1.	ALKALOIDS					
	Mayer's reagent	+	-	+	+	-
	Dragendroff's reagent	+	-	+	+	-
2.	GLYCOSIDES					
	Killer-Killani test	-	-	-	-	-
	Sodium nitropruside test	-	-	-	-	-
	Borntrager test	-	+	-	+	-

RESULTS AND DISCUSSION

Sr. No.	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chlorofor m extract	Ethanol extract	Aqueous extract
3.	CARBOHYDRATES Molisch's reagent Fehling solution	- -	- -	- -	+	+
4.	STEROLS Liebermann- Burchard's test Salkowski test Hesses reaction Hersch reaction	- - + -	- - - -	- - - -	+	- - - -
5.	SAPONINS Foam test Sodium bicarbonate test	- -	- -	- -	+	- -
6.	PHENOLIC COMPOUNDS & TANNINS Ferric chloride solution Lead acetate solution	- -	- -	- -	- +	- -
7.	PROTEINS & AMINO ACIDS Biuret test Millon's reagent Ninhydrin reagent	- - +	- - -	- + +	+	- - +
8.	FLAVANOIDS Shinoda/Pew test Ammonia test	- -	- -	- -	+	- -

+ve : Detected; -ve : absent

3.1.13 Discussion

***Murraya koenigii* roots:** The phytochemical and pharmacognostical evaluation of *Murraya koenigii* roots was performed. The root is a typical root and in transverse section it shows the features of a dicot root, i.e. Epidermis, Cortex, Endodermis, Phloem and Xylem. The microscopic property of the powder shown xylem vessel; fiber; parenchyma cell and cork cells. The Total ash; water insoluble ash; sulphated ash; and acid insoluble ash (11.25%; 8.75%; 7.025%; 10.57%). Extracting values, i.e. petroleum ether (PE); chloroform (CF); ethyl acetate (EA); ethanol (ET) and aqueous extract (3.5 %; 5.3 %; 3.2%; 8.5% 4.5%). The fiber content was found to be 7.53%. Plant bitterness was found to be 2.5 unit/gm. The plant also has hemolytic potential. The plant extracts were free of microbial contamination. They do not show any microbial growth in the specified nutrient media. The tannin content was found to be

RESULTS AND DISCUSSION

19. The water extract was screened for the presence of different amino acids and carbohydrates. The extract showed the presence of two amino acids viz. Phenylalanine and glycerin and two carbohydrates i.e. galactose, Ribose and fructose. The preliminary phytochemical screening of petroleum ether, ethyl acetate, chloroform, ethanol and aqueous extract was used. The phytochemical screening showed presence of alkaloids; carbohydrates; flavonoids and sterol in different extracts were practically observed.

***Ficus lacor* aerial roots:** The pharmacognostical investigations i.e. morphological study of the aerial root of the plant was performed. The aerial roots are typical roots and in transverse section (T.S) it shows the characteristics of a dicot root. The microscopic characteristics of the powder showed the presence of the annular xylem vessel; lignified fiber; parenchyma cell and cork cells. The total ash; water insoluble; sulphated ash and Acid insoluble ash (14.15%; 10.75%; 6% and 8.57%). Petroleum ether extractive; chloroform extractive; ethyl acetate extractive; ethanol extractive and aqueous extractive (5.7%; 10%; 5.5%; 4.5%; 10.5%). The fiber content was found to be 9.45 percent. The plant bitterness was found to be 1.9 unit/gm. The foaming index was found to be 124.6. The plant have haemolytic potential. The tannin content was 22. The alcohol and water extracts were screened for presence of amino acids and carbohydrates. The three amino acid viz. Tyrosine; Alanine and Methionine and three carbohydrates i.e. Sucrose Galactose and Lactose. The preliminary phytochemical investigations showed the presence of carbohydrates; flavonoids; phenolic compounds; saponins and sterol in different extracts were practically observed.

3.2 Column Chromatography

3.2.1 Compounds Isolated by Column Chromatography

Compounds RKFL-I

Elution of the column with petroleum ether : chloroform (19 : 1) fraction no. 44-72 (flask no. 9-13) gave colourless crystals of compound **RKFL-I** recrystallized from pure ethyl acetate 254.70 mg (0.254% yield). R_f : 0.58 (petroleum ether : chloroform :: 19 :1), m.p. 65-70°C.

UV λ_{max} (Methanol) : 217 nm

IR ν_{max} (KBr) : 2916, 2850, 1729, 1635, 1468, 1380, 1361, 1264, 1198, 1174, 1096, 989, 968, 717 cm^{-1} .

$^1\text{H NMR}$ (CDCl_3), $^{13}\text{C NMR}$ (CDCl_3) shown below:

RESULTS AND DISCUSSION

¹H NMR and ¹³C NMR spectral data of RKFL-I:

Position of Carbon	¹H NMR		¹³C NMR
	Alpha	Beta	
1	1.96 ddd (<i>J</i> = 4, 8.8, 8.8)	1.34 ddd (<i>J</i> = 5.2, 8.8, 4.4)	38.30
2	1.60 ddd (<i>J</i> = 4.8, 5.5, 8.8)	1.63 ddd (<i>J</i> = 5.5, 3.7, 7.6)	23.58
3	4.50 dd (<i>J</i> = 5.5, 8.8)	-	80.63
4	-	-	37.82
5	1.57 dd (<i>J</i> = 5.6, 6.8)	-	55.30
6	1.42 dd (<i>J</i> = 9.2, 5.6)	1.61 m	18.21
7	1.35 dd (<i>J</i> = 4.4, 9.2)	1.04 dd (<i>J</i> = 4.4, 2.0)	34.96
8	-	-	41.76
9	1.66 m	-	48.70
10	-	-	36.90
11	1.86 dd (<i>J</i> = 3.6, 6.1)	1.58 dd (<i>J</i> = 5.6, 3.6)	23.61
12	5.18 dd (<i>J</i> = 3.6, 3.6)	-	121.71
13	-	-	145.29
14	-	-	39.86
15	1.32 dd (<i>J</i> = 4, 10.1)	1.34 dd (<i>J</i> = 5.2, 4.0)	26.20
16	1.29 dd (<i>J</i> = 4.4, 10.1)	1.07 dd (<i>J</i> = 2.8, 4.4)	26.98
17	-	-	32.02
18	-	1.60 ddd (<i>J</i> = 4.8, 5.5, 8.8)	47.26
19	1.08 dd (<i>J</i> = 2.8, 9.6)	1.11 dd (<i>J</i> = 6.8, 2.8)	47.60
20	-	-	31.17
21	1.39 dd (<i>J</i> = 3.2, 10.4)	1.28 m	32.64
22	-	-	34.79
23	0.85 br s	-	28.49
24	0.94 br s	-	16.13
25	0.96 br s	-	17.15
26	0.88 br s	-	23.68
27	0.89 br s	-	23.77
28	0.82 br s	-	28.13
29	0.96 br s	-	33.43
30	1.01 br s	-	26.05

Position of Carbon	¹H NMR		¹³C NMR
	Alpha	Beta	
1'	-	-	173.81

RESULTS AND DISCUSSION

2'	2.31 d (<i>J</i> = 7.2)	2.27 d (<i>J</i> = 8.0)	46.84
3'	1.27 m	1.27 m	37.21
4'	1.25 br s	1.25 br s	32.57
5'	1.25 br s	1.25 br s	29.78
6'	1.25 br s	1.25 br s	29.78
7'	1.23 br s	1.23 br s	29.67
8'	1.23 br s	1.23 br s	29.57
9'	1.13 br s	1.13 br s	29.47
10'	1.17 br s	1.17 br s	29.36
11'	1.10 br s	1.10 br s	29.27
12'	1.10 br s	1.10 br s	29.26
13'	1.10 br s	1.10 br s	29.16
14'	-	-	29.58
15'	1.10 br s	1.10 br s	22.63
16'	0.84 t (<i>J</i> = 6.1)	-	14.65

Coupling constants in Hertz are provided in parenthesis.

ESMS *m/z* (rel. int.) : 664 [M]⁺ (C₄₆H₈₀O₂) (1.3), 425 (8.3), 393 (6.8), 256 (11.5), 218 (12.3), 206 (5.8), 175 (39.7), 159 (15.6)

Compounds RKFL-II

Elution of the column with petroleum ether : chloroform (1 : 1), furnished colourless crystals of **RKFL-II**, recrystallized from acetone, 302 mg (0.30% yield), R_f = 0.60 (Pet. ether : Chloroform :: 1:1), m.p. 55-58°C.

UV λ_{\max} (Methanol) : 238 nm (log E5-1)

IR ν_{\max} (KBr) : 2914, 2848, 1728, 1635, 1469, 1270, 1208, 1173, 1112, 1036, 889, 718 cm⁻¹.

¹H NMR (CDCl₃), **¹³C NMR** (CDCl₃)

ESMS *m/z* (rel. int.) : 692 [M]⁺ (C₄₈H₈₄O₂) (1.3), 425 (6.2), 284 (12.1), 218 (5.2), 207 (3.5)

¹H NMR and ¹³C NMR spectral data of RKFL-II :

Position of Carbon	¹ H NMR		¹³ C NMR
	Alpha	Beta	
1	1.95 dd (<i>J</i> = 4, 8.4)	1.31 dd (<i>J</i> = 3.6, 4.0)	38.32

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2	1.60 ddd (<i>J</i> = 8.4, 2.8, 4.0)	1.63 ddd (<i>J</i> = 2.8, 7.3, 2.8)	23.54
3	4.50 dd (<i>J</i> = 5.3, 8.9)	-	80.63
4	-	-	37.83
5	1.55 dd (<i>J</i> = 6.8, 4.1)	-	55.32
6	1.42 m	1.62 m	18.34
7	1.35 dd (<i>J</i> = 2.4, 10.0)	1.03 dd (<i>J</i> = 2.4, 2.4)	34.94
8	-	-	41.78
9	1.66 ddd (<i>J</i> = 10.4, 2.8, 7.6)	-	47.62
10	-	-	36.92
11	1.86 dd (<i>J</i> = 8.4, 2.4)	1.58 dd (<i>J</i> = 2.8, 4.0)	23.61
12	5.18 dd (<i>J</i> = 4.0, 3.7)	-	121.72
13	-	-	145.28
14	-	-	39.88
15	1.29 dd (<i>J</i> = 4.0, 7.6)	1.31 dd (<i>J</i> = 3.6, 4.0)	26.21
16	1.33 dd (<i>J</i> = 10.1, 5.2)	1.03 dd (<i>J</i> = 2.4, 4.4)	27.00
17	-	-	32.02
18	-	1.61 ddd (<i>J</i> = 2.8, 7.6, 2.8)	47.30
19	1.08 dd (<i>J</i> = 6.4, 2.8)	1.11 dd (2.8, 2.8)	47.31
20	-	-	31.16
21	1.34 dd (<i>J</i> = 2.4, 10.1)	1.28 m	32.66
22	1.39 dd (<i>J</i> = 10.1, 3.2)	1.43 dd (<i>J</i> = 3.2, 6.4)	34.81
23	0.86 br s	-	28.48
24	0.94 br s	-	15.63
25	0.96 br s	-	16.86
26	0.88 br s	-	23.68
27	0.89 br s	-	23.77
28	0.82 br s	-	28.13
29	0.94 br s	-	33.41
30	1.13 br s	-	26.04

Position of Carbon	¹ H NMR		¹³ C NMR
	Alpha	Beta	
1'	-	-	173.76
2'	2.3 d (<i>J</i> = 7.3)	2.27 d (<i>J</i> = 7.6)	46.86
3'	1.27 br s	1.27 br s	37.22
4'	1.26 br s	1.26 br s	32.57
5'	1.26 br s	1.26 br s	29.77
6'	1.26 br s	1.26 br s	29.77
7'	1.26 br s	1.26 br s	29.77
8'	1.25 br s	1.25 br s	29.68
9'	1.25 br s	1.25 br s	29.68
10'	1.25 br s	1.25 br s	29.68

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11'	1.25 br s	1.25 br s	29.56
12'	1.23 br s	1.23 br s	29.46
13'	1.23 br s	1.23 br s	29.35
14'	1.23 br s	1.23 br s	29.26
15'	1.25 br s	1.23 br s	25.24
16'	1.23 br s	1.23 br s	25.24
17'	1.19 br s	1.19 br s	22.78
18'	0.84 t (<i>J</i> =6.0)	-	14.21

Coupling constants in Hertz are provided in parenthesis.

Compound RKFL- III

Elution of the column with chloroform yielded colourless crystals of **RKFL- III** recrystallized from methanol, 82.80 mg (0.082%), $R_f = 0.56$ (chloroform), m.p. 62-65°C.

UV λ_{\max} (Methanol) : 201 nm

IR ν_{\max} (KBr) : 2919, 2850, 1740, 1464, 1375, 1266, 1168, 723 cm^{-1} .

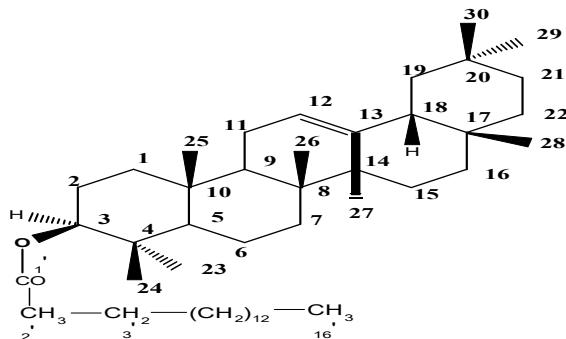
$^1\text{H NMR}$ (DMSO-*d*6) : δ 302 (2H, br s, H₂-1'), 2.27 (1H, d, *J* = 7.2 Hz, H₂-2a), 2.25 (1H, d, *J* = 7.2 Hz, H₂-2b), 2.03 (2H, m, H₂-3), 1.52 (2H, m, H₂-2'). 129 (6H br s, 3 x CH₂), 1.25 (56 H, br s, 28x CH₂) 0.88 (3H, t, *J* = 5.6 Hz Me-14), 0.84 (3H, t, *J* = 6.8 Hz, Me-24').

$^{13}\text{C NMR}$ (CDCl₃) : δ 172.98 (C-1), 68.41 (C-1'), 32.81 (CH₂), 29.60 (30 x CH₂), 22.27 (2 x CH₂), 22.61 (CH₂), 14.19 (Me-14), 14.16 (Me-24').

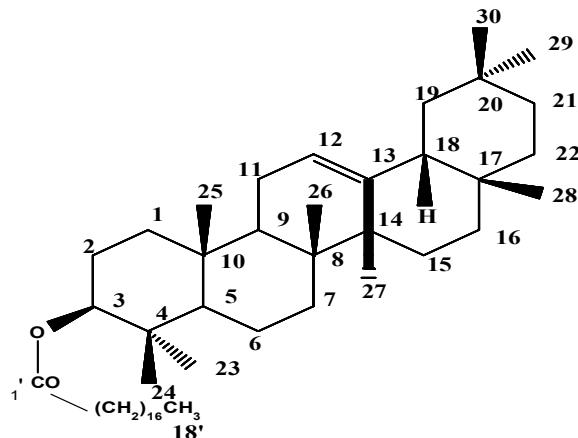
ESMS m/z (rel. int.) : 564 [M]⁺ (C₃₈H₇₆O₂) (13.1), 353 (63.8), 337 (8.5), 308 (8.2), 227 (7.6), 212 (7.9), 211 (27.7).

Compound RKFL-I

On the basis of foregoing discussion the structure of RKFL-I has been elucidated as olean-18- β -H-12-en-3- β -yl hexadecanoate.



This is a new pentacyclic triterpene ester isolated from a natural source.

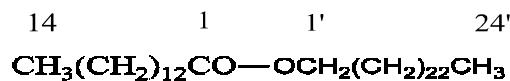
Compound RKFL-II

On the basis of spectral data analysis and chemical reaction the structure of RKFL-II has been established as olean-12-en-3 β yl n-octadecanoate.

Compound RKFL-III

On the basis of above discussion, the structure RKFL-III has been characterized as n-tetracosanyl, n-tetradecanoate.

This is the first report of occurrence of a fatty acid ester in *Ficus lacor*



The various terpenoidal fractions separated by column, mixed together and named as FLTP-1. Then, screened for pharmacological activities.

Table 3.11 A: Physical constants and nomenclature of chemical constituents isolated from *Ficus lacor* aerial roots

Code No.	Common Name, Molecular formula, Molecular weight	Column fraction R_f value	%age yield	Melting point	Nomenclature (Remarks)
RKFL-I	β -amyrin palmitate ($C_{46}H_{80}O_2$) (664)	Petroleum ether : Chloroform (19:1) (0.58)	0.254	65-70°C	olean-18- β -H-12-en-3- β -yl hexadecanoate [New]
RKFL-II	β -amyrin stearate ($C_{48}H_{84}O_2$) (692)	Petroleum ether : Chloroform (1:1) (0.60)	0.302	55-58°C	olean-12-en-3- β -yl n-octadecanoate [New]
RKFL-III	Myristyl tetradecanoate $C_{38}H_{76}O_2$ (564)	Chloroform (0.56)	0.082	62-65°C	n-tetracosanyl, n-tetradecanoate [New]

3.2.2 Compounds isolated by column chromatography from *Murraya koenigii*

RKMK-1

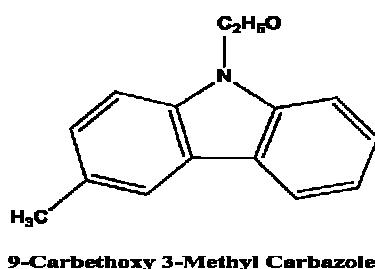
Elution of the column with petroleum ether fraction number 19-30 gave colorless needle shape RKMK-1, R_f 0.84 in petroleum ether (0.32% yield), m.p. 121-123°C.

UV λ_{max} (Methanol): 229, 251, 252, 262, 277, 286, 304, 314

IR ν_{max} (KBr) : 1718, 1482, 1441, 1373, 1341, 1320, 1300, 1250, 1223, 1044, 800.

ESMS m/z (rel. int.): 253[M]⁺ 71, 193(44), 181(55), 180(100), 152(18)

RKMK-1, the alkaloid of intermediate polarity, was identified by thorough spectral studies as 9-Carbethoxy-3-methylcarbazole (Guangzhi *et al.*, 1980).



RKMK-2

Elution of the column with petroleum ether: choloroform (3:1) fraction number 31-60 gave white greasy mass of RKMK-2, R_f 0.75 in petroleum ether: choloform (4:1) (0.184% yield), m.p. 205-207°C (Chowdhury *et al.* 1987).

UV λ_{max} (Methanol): 3407, 1607, 1495, 1475, 807, 749, 729.

IR ν_{max} (KBr) : 3407, 2915, 1607, 1495, 1475, 1461, 335, 243, 807, 749, 729, 592, 573, 454.

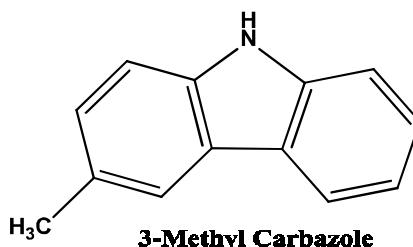
¹H NMR (DMSO-d₆): (270 MHz), 8.04 (d, J = 7.91 Hz, H-5), 7.94 (s, N-H), 7.87 (s, H-4), 7.40 (d, J = 8.16 Hz, H-1 and H-8), 7.32 (d, J = 8.16 Hz, H-2), 7.21 (m, H-6 and H-7), 2.53 (s, CH₃-3)

¹³C NMR (CDCl₃): δppm (67.4, CDCl₃); 139.8 (Carbon-1 a), 137.8 (Carbon-8a), 128.7 (Carbon-5a), 127.1 (Carbon-1), 125.6 (Carbon-7), 123.5 (Carbon-4a), 123.2 (Carbon-3), 120.2 (Carbon-4, C-5), 119.2 (Carbon-2), 110.5 (Carbon-8), 110.2 (Carbon-6), 21.4, (Carbon-9)

ESMS m/z (rel. int.): 181 (M⁺, 100), 180 (78), 179(6), 178(5), 153(3), 152 (8), 90 (23), 77 (7), 6(4), 63(3), 51(1)

RESULTS AND DISCUSSION

The compound was elucidated as 3-methylcarbazole (RKMK-2), which was isolated previously from Clausena heptaphylla and Glycosmis pentaphylla (Chowdhury *et al.* 1987; Sukari *et al.*, 2001).



RKMK-3

Elution of the column with Chloroform: Petroleum ether (19: 1) fraction number 127-155 gave white mass of RKMK-3, R_f value 0.22 in chloroform: petroleum ether (5:2) (0.06% yield), m.p. 129-130°C.

UV λ_{\max} (Methanol): 334 (3.55), 323 (3.60), 287 (4.03), 279 (3.86), 259 (4.39), 251 (4.22), 241 (4.70), 225 (4.57)

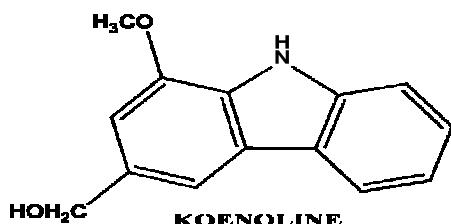
IR ν_{\max} (KBr) : 3445, 3235, 1585, 1500, 1450, 1390, 1335, 1310, 1280, 1260, 1225, 1130, 1100, 1035, 1010, 955, 940, 830, 735

¹H NMR (DMSO-d6): HNMR (360 MHz): δ1.75 (1H, *brs*, exchange with D₂O, hydroxyl group), 4.01 (3H, s, Methoxy), 4.84 (2H, s, Ar-CH₂OH), 6.95 (1H, *brs*, H-2), 7.23 (1H, ddd, J = 8.0, 7.0, 1.3 Hz, H-6), 7.42 (1H, ddd, J= 8.0, 7.0, 1.2 Hz H-7), 7.46 (1H, *d*, J = 8.0 HZ, H-8), 7.66 (1H, *brs*, H-4), 8.04 (1H, *d*, J = 8.0Hz, H-5)and 8.34ppm (1H, *brs*, exchange with D₂O, NH)

¹³C NMR (CDCl₃): 111, 125.6, 118.7, 120.10, 120.1, 118.8, 125.7, 111.1, 139.9, 122.6, 122.7, 139.10

ESMS m/z (rel. int.): 227[M]⁺ (100, 212 (28), 210(75), 198 (25), 183 (23), 167 (45), 154 (30), 139 (14), 127 (15), 113 (7), 99 (14), 77 (14)

The ¹³C NMR data of RKMK3 were assigned with reference to previous studies by (Gribble *et al.*, 1975 and Ahond *et al.* 1978). RKMK-3 molecular formula of C₁₄H₁₃NO₂, obtained by mass spectrometry. RKMK-3, was identified by thorough spectral studies as Koenoline.



RESULTS AND DISCUSSION

The various alkaloidal fractions separated by column, mixed together them and named as MKAF-1. Then, screened for pharmacological activities.

Table 3.11 B: Physical constants and nomenclature of chemical constituents isolated from *Murraya koenigii* roots

Code No.	Common Name, Molecular formula, Molecular weight	Column fraction R_f value	%age yield	Melting point	Nomenclature (Remarks)
RKMK-1	9-carbethoxy-3-methyl carbazole (C ₁₆ H ₁₅ NO ₂) (253.30)	Petroleum ether (0.84)	0.320	121-123°C	9-carbethoxy-3-methyl carbazole [Reported]
RKMK-2	3-methyl carbazole C ₁₃ H ₁₁ N (181.3)	Petroleum ether : Chloroform (3:1) (0.75)	0.184	205-207°C	3-methyl carbazole [Reported]
RKMK-3	Koenoline (C ₁₄ H ₁₃ NO ₂) (227.26)	Chloroform: Petroleum ether (19: 1) (0.22)	0.062	129-130°C	1-methoxy-3-hydroxy methyl carbazole [Reported]

3.3 Pharmacological screening:

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

3.3.1 Screening for Anti-inflammatory Activity

3.3.1.1 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 tables 3. 12 A & B.

3. 12 A: Inhibition of Carragenan induced paw edema in rats by diffrent 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.

3.12 B: 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 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).

RESULTS AND DISCUSSION

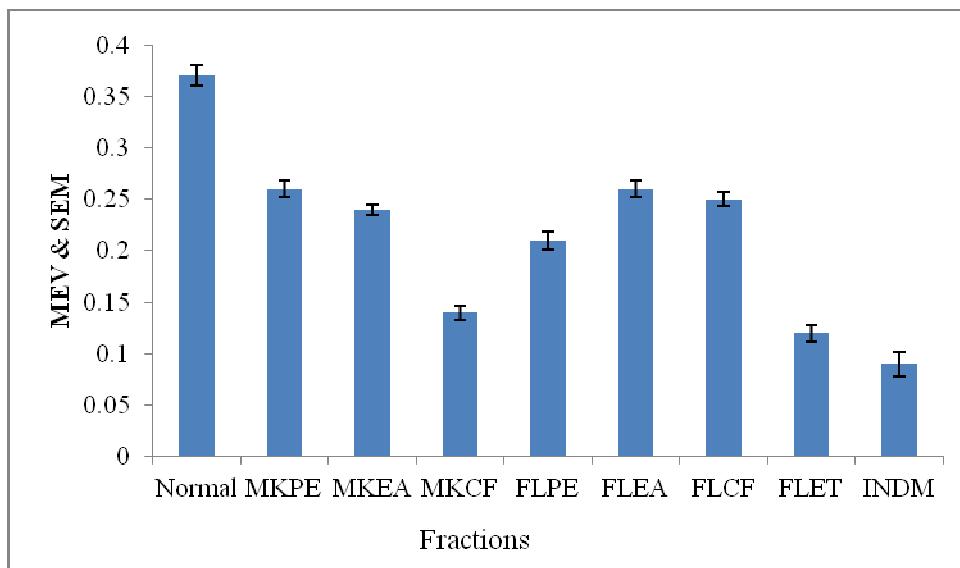


Figure 3.7: Inhibition of Carragenan induced paw edema in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 50mg/kg, body weight

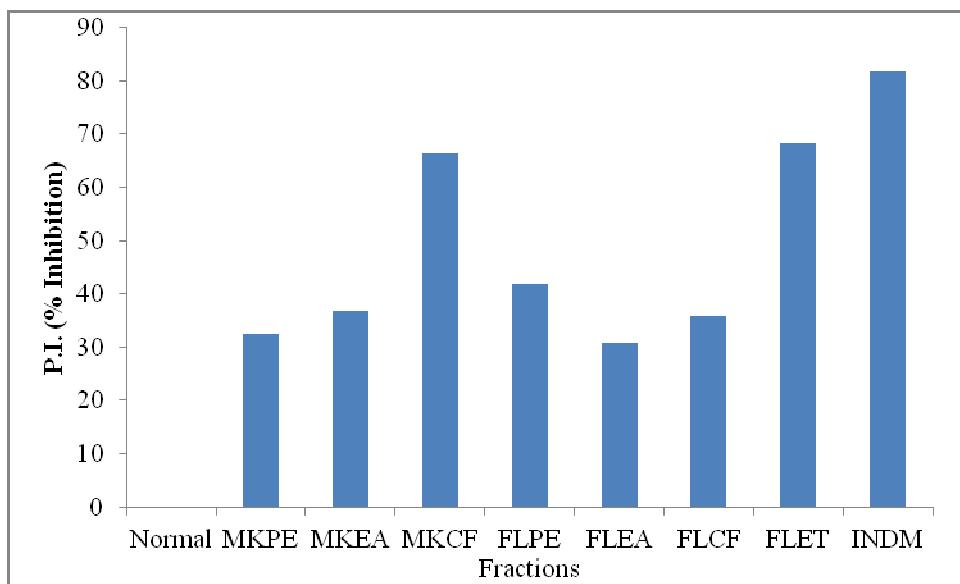


Figure 3.8: Percentage Inhibition of Carrageenan induced edema in rats by various fractions isolated from roots *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 50mg/kg body weight

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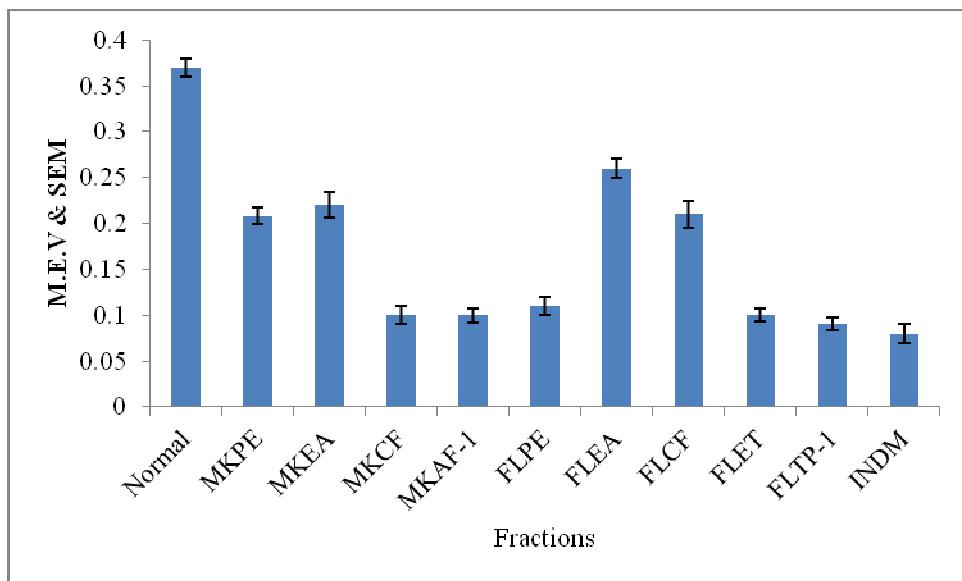


Figure 3.9: Inhibition of Carrageenan induced edema paw edema in rats by various fractions isolated from roots *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 100mg/kg body weight

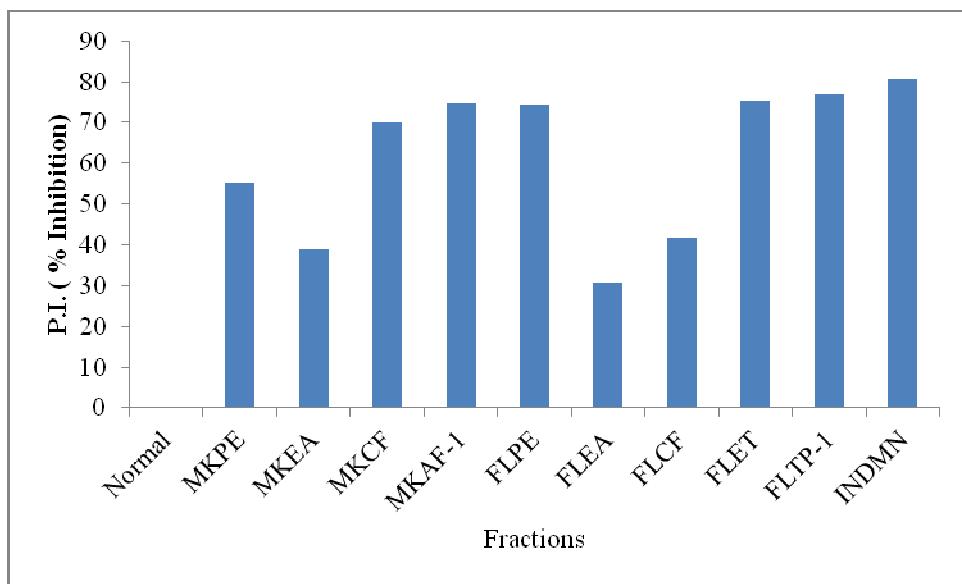


Figure 3.10: Percentage Inhibition of paw edema, induced via Carrageenan in rats by various fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at the dose of 100mg/kg

3.1.2 Inhibition of histamine induced paw edema in rats

3. 13 A: Inhibition of paw edema, induced via histamine in rats by various fractions from roots of *Murraya koenigii* and aerial roots of *Ficus lacor*

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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 \pm 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.

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3. 13 B: 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 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.

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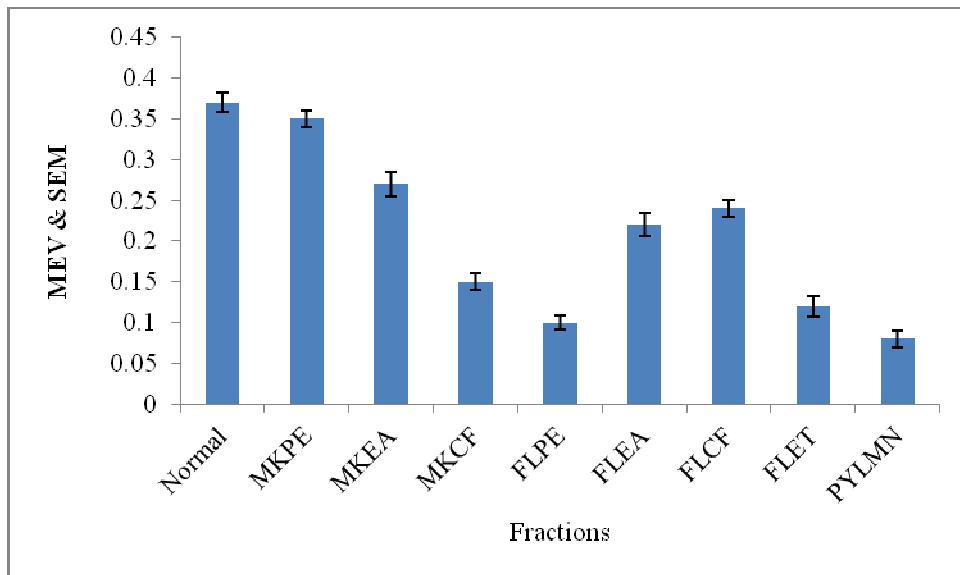


Figure 3.11: Inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 50mg/kg body weight

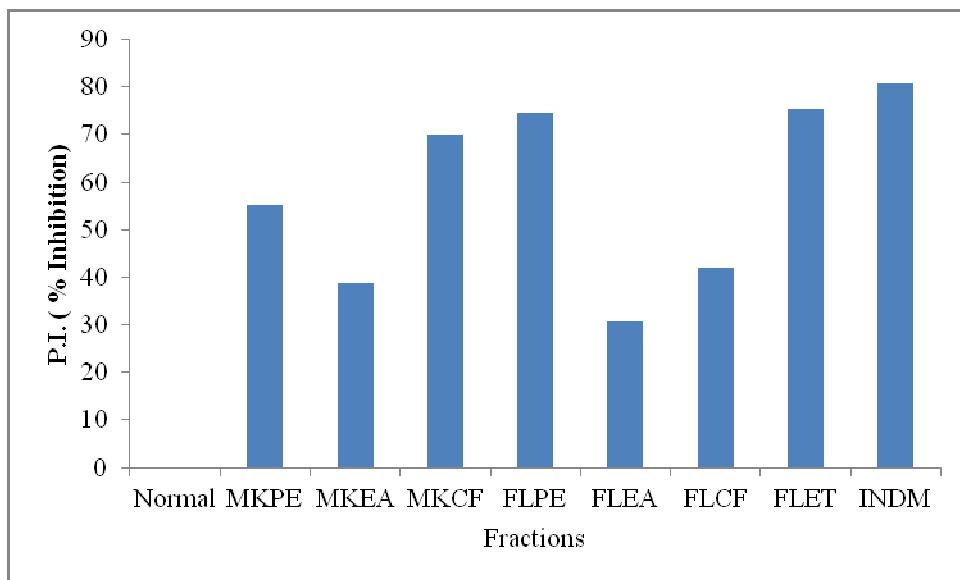


Figure 3.12: Percentage Inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 50mg/kg body weight

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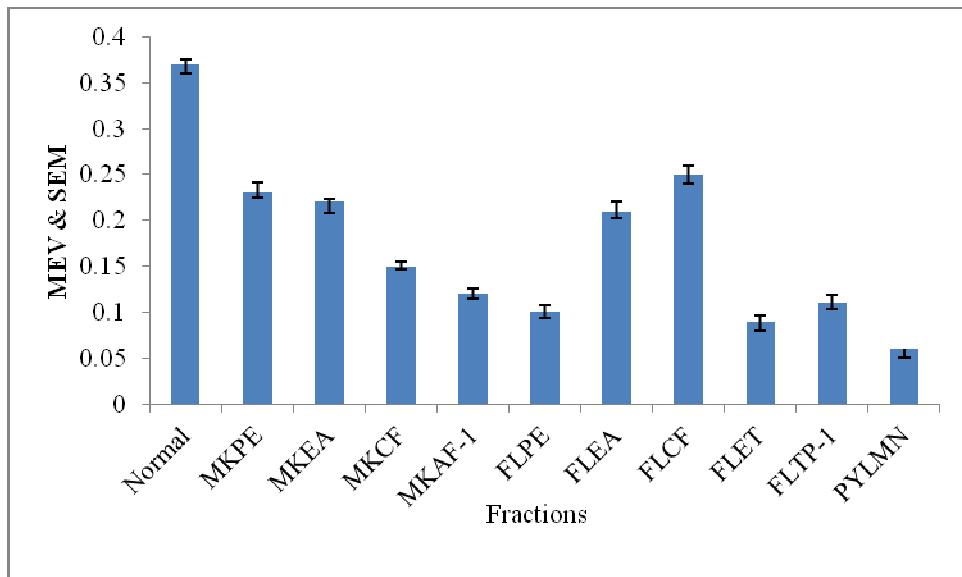


Figure 3.13: Inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 100mg/kg body weight

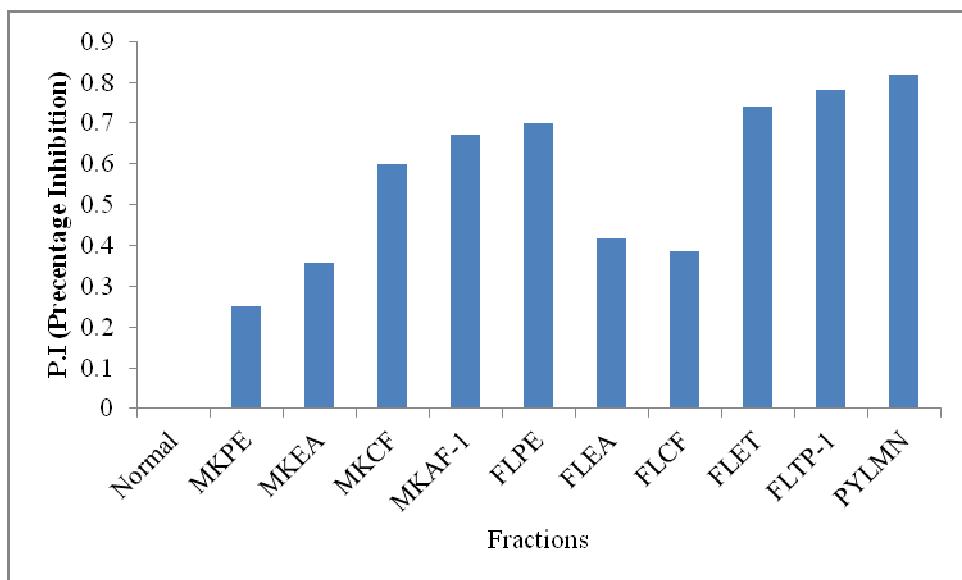


Figure 3.14: Percentage inhibition of paw edema, induced via histamine in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 100mg/kg body weight.

3.3.1.3 Inhibition of Serotonin induced paw edema in rats

3. 14 A: Inhibition of Serotonin induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Groups	Isolated Fractions	Dose mg/kg	Serotonin	
			MEV	PI
I.	Normal	(Normal saline 1ml) Arthritic control	0.53±0.04	--
II.	MKPE	50	0.46±0.02	14.14
III.	MKEA	50	0.42±0.02*	22.64
IV.	MKCF	50	0.38±0.02*	31.64
V.	FLPE	50	0.13±0.01***	69.10
VI.	FLEA	50	0.41±0.02 *	22.64
VII.	FLCF	50	0.39±0.07*	28.64
VIII.	FLET	50	0.13±0.01**	66.1
IX	Pyrilamine	1mg/kg	0.12±0.01***	80.1

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.

3. 14 B: Inhibition of Serotonin induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Serotonin	
			MEV	PI
I	Normal	(Normal saline 1ml) Arthritic control	0.53±0.04	--
II.	MKPE	100	0.43±0.02	18.14
III.	MKEA	100	0.40±0.02*	22.60
IV.	MKCF	100	0.38±0.02*	31.32
V.	MKAf-1	100	0.25±0.02*	52.85
VI.	FLPE	100	0.20±0.01***	62.72
VII.	FLEA	100	0.41±0.02 *	22.64
VIII.	FLCF	100	0.40±0.07*	28.14
IX.	FLET	100	0.16±0.01***	68.72
X	FLTP-1	100	0.14±0.01***	73.58
XI	Pyrilamine	1mg/kg	0.09±0.01***	80.01

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, 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 FLET, isolated from *Ficus lacor*, Group XI- Treated with standard drug Pyrilamine at the dose of 1mg/kg body weight; *p<0.05; as compared with arthritic control.

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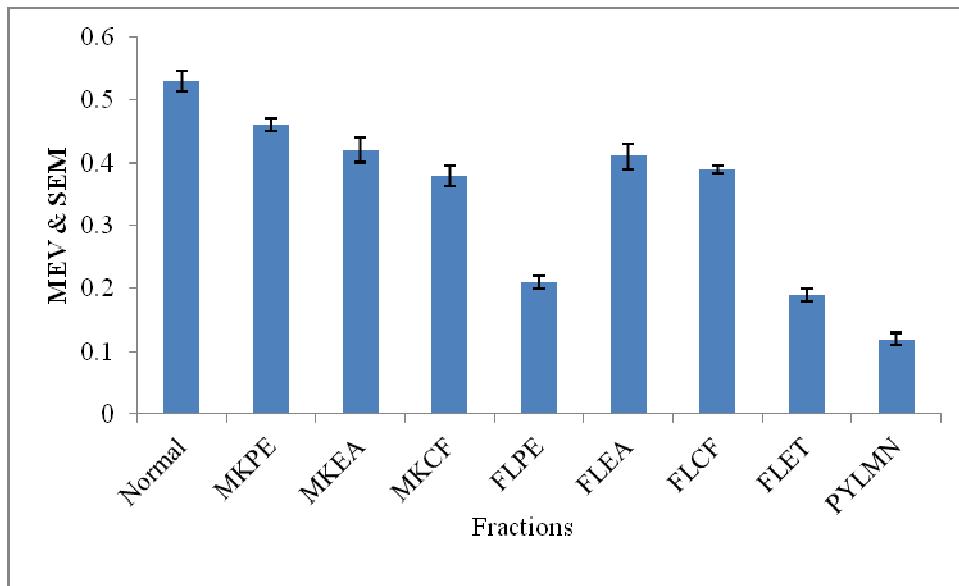


Figure 3.15: Inhibition of paw edema in rats induced via Serotonin, by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 50mg/kg body weight

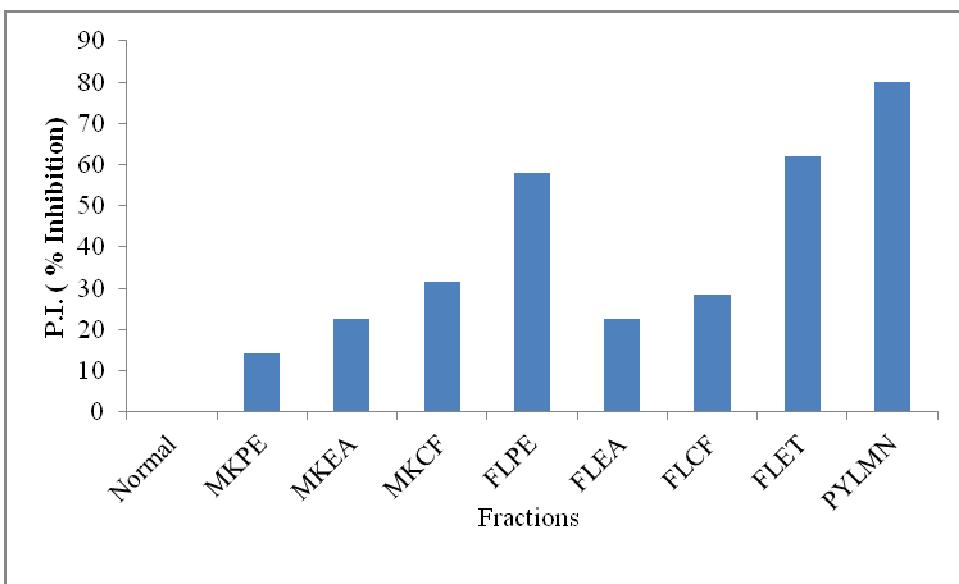


Figure 3.16: Percentage Inhibition of paw edema in rats induced via Serotonin, by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at 50mg/kg body weight

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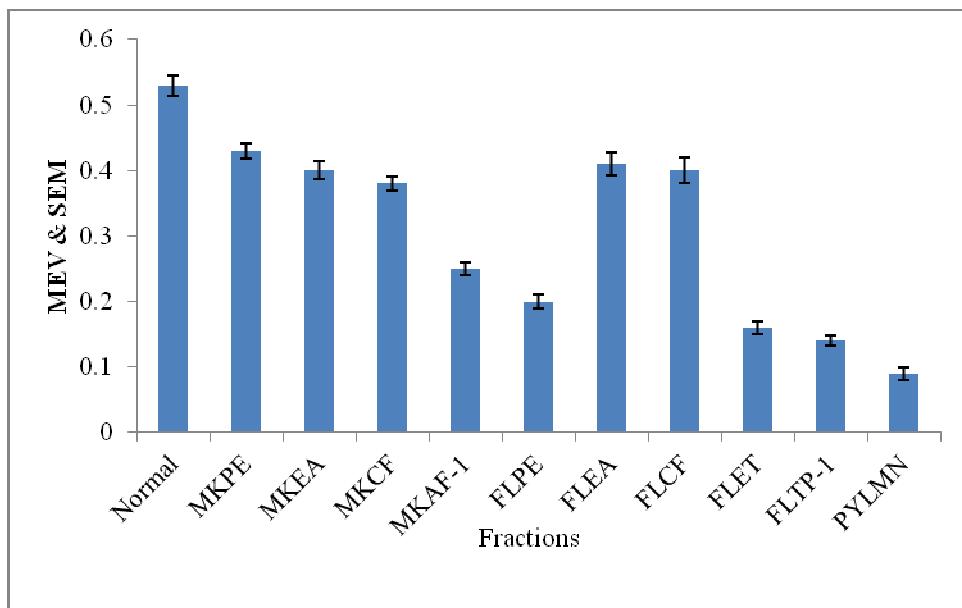


Figure 3.17: Inhibition of paw edema in rats induced via Serotonin, by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 100mg/kg body weight

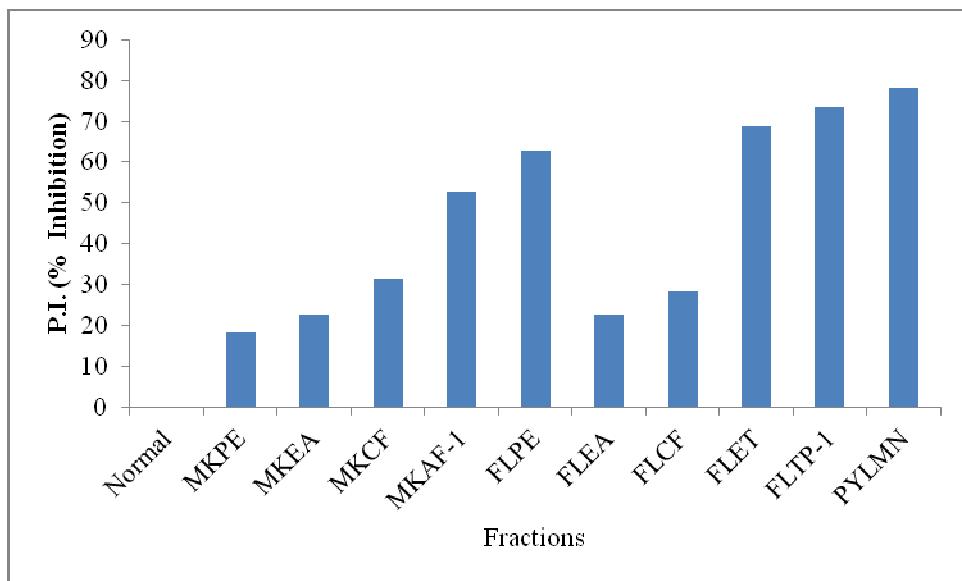


Figure 3.18: Percentage Inhibition of Serotonin induced paw edema in rats by different fractions isolated from roots of *Murraya koenigii* and aerial roots of *Ficus lacor* at dose of 100mg/kg body weight

3.3.1.4 Inhibition of formalin induced paw edema in rats

The separated fractions from the two plants were screened for their capacity to inhibit formalin induced paw edema in rats at different doses given intraperitoneally. The observed inhibitions are tabulated in Tables 3.15 A to 3.15 B

3. 15 A: Inhibition of formalin induced paw edema in rats by various extracts from *Murraya koenigii* and *Ficus lacor*

Groups	Isolated Extracts	Dose mg/kg	MEV & PI		
			1h	2h	4h
I.	Normal	(Normal saline 1ml) Arthritic control	0.54±0.05 37.34%	0.65±0.07 38.47%	0.72±0.04
II.	MKPE	50mg	0.34±0.03** 37.34%	0.40±0.03** 38.47%	0.48±0.05** 34.33%
III.	MKEA	50mg	0.48±0.05 11.13%	0.57±0.04 10.76%	0.64±0.04 11.11
IV.	MKCF	50mg	0.32±0.04** 40.75%	0.37±0.04** 43.08%	0.39±0.03** 45.84%
V.	FLPE	50mg	0.30±0.03** 44.46%	0.35±0.04** 46.16%	0.42±0.05** 40.00%
VI.	FLEA	50mg	0.42±0.10 22.24%	0.49±0.04** 24.62%	0.54±0.05* 25.00%
VII.	FLCF	50mg	0.49±0.05 12.96%	0.56±0.04 13.85%	0.63±0.04 12.50
VIII.	FLET	50 mg	0.28±0.02** * 48.15%	0.32±0.02** 50.77%	0.33±0.05** 54.17
IX	Diclofenac	5 mg	0.30±0.02** 51.85%	0.28±0.04*** 53.85%	0.23±0.03*** 65.28%

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 Diclofenac 5mg/kg; *p<0.05; **p<0.01; *** p <0.001, as compared with arthritic control.

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3. 15 B: Inhibition of formalin induced paw edema in rats by various extracts from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Extracts	Dose ml/kg mg/kg	MEV & PI		
			1h	2h	4h
I	Normal	(Normal saline 1ml) Arthritic control	0.54±0.05	0.65±0.07	0.72±0.04
II.	MKPE	100	0.31±0.04** 42.60%	0.36±0.03** 44.16%	0.40±0.05** 44.46%
III.	MKEA	100	0.48±0.05 12.03%	0.57±0.04 12.30%	0.64±0.06 11.53
IV.	MKCF	100	0.31±0.06** 42.61%	0.34±0.03** 47.78%	0.39±0.03** 45.84%
V.	MKAf-1	100	0.32±0.043** * 59.45%	0.32±0.03** 50%	0.35±0.03** 51.38%
VI.	FLPE	100	0.30±0.03** 46.30%	0.35±0.04** 46.16%	0.36±0.06** 50.01%
VII.	FLEA	100	0.42±0.07* 22.24%	0.49±0.04** 24.62%	0.54±0.05* 25.02%
VIII.	FLCF	100	0.49±0.05 09.63%	0.57±0.04 10.76%	0.64±0.04 11.10
IX.	FLET	100	0.26±0.03*** 50.95%	0.31±0.04** 52.30%	0.39±0.05** 55.57
X	FLTP-1	100	0.32±0.03*** 40.75%	0.30±0.03*** 53.84%	0.33±0.03*** 58.17
XI	Diclofen	5 mg	0.30±0.02** 51.85%	0.28±0.04*** 53.85%	0.23±0.03*** 65.28%

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, 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 Diclofen 5mg/kg, *p<0.05; **p<0.01; *** p <0.001, as compared with arthritic control.

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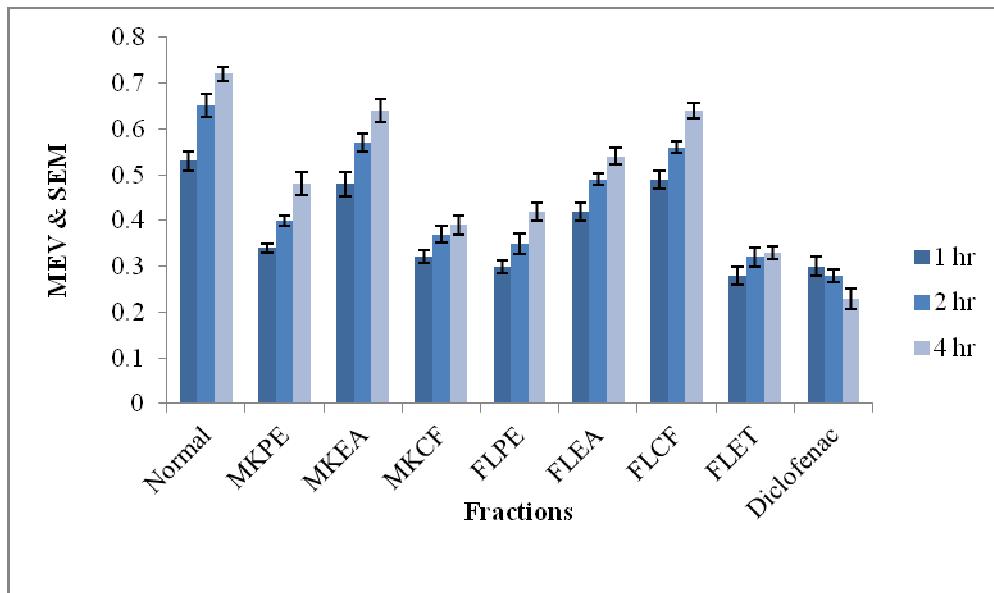


Figure 3.19: Inhibition of formalin induced paw edema in rats by various extracts from *Murraya koenigii* and *Ficus lacor* at 50mg/kg body weight

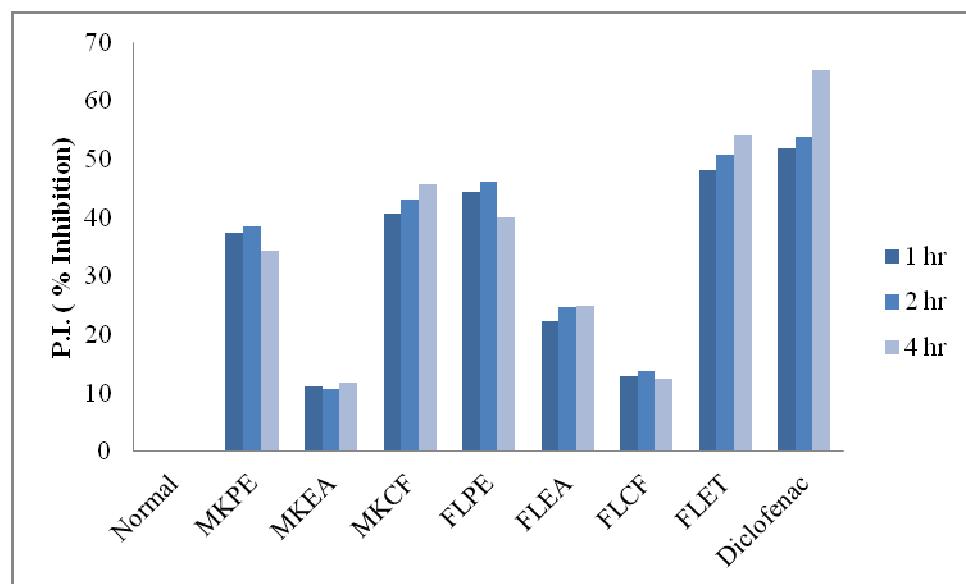


Figure 3.20: Percentage Inhibition of formalin induced paw edema in rats by various extracts from *Murraya koenigii* and *Ficus lacor* at 50mg/kg body weight

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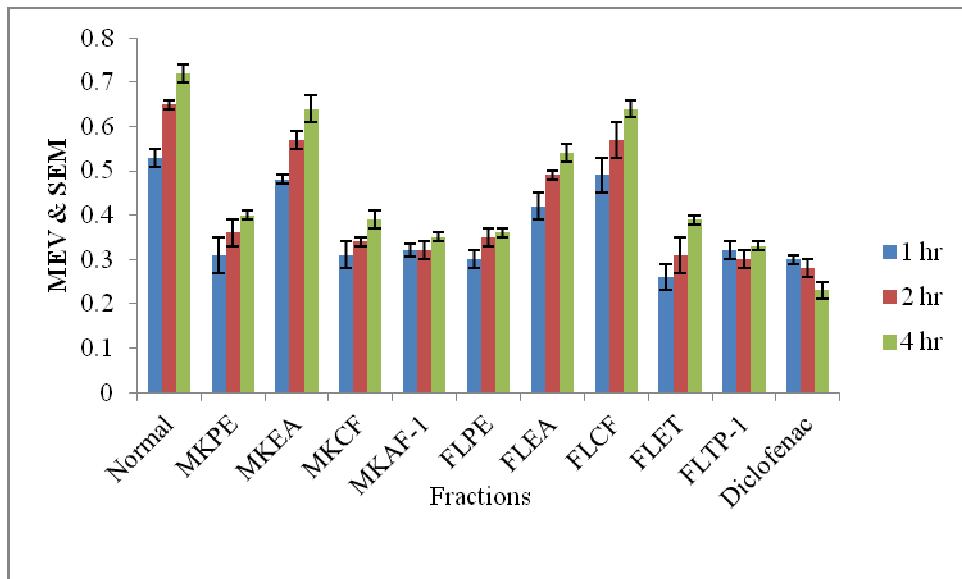


Figure 3.21: Inhibition of formalin induced paw edema in rats by various extracts from *Murraya koenigii* and *Ficus lacor* at 100mg/kg body weight

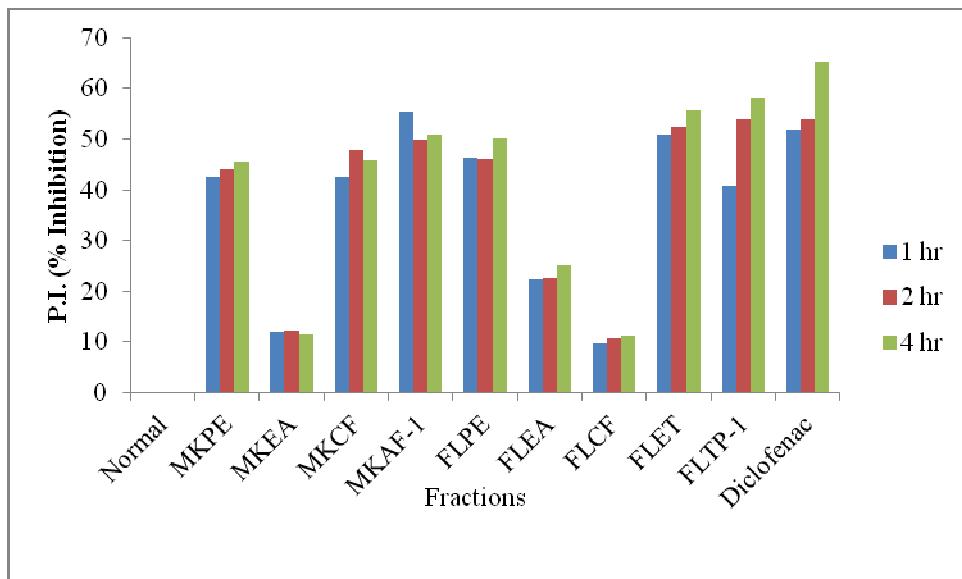


Figure 3.22: Percentage Inhibition of formalin induced paw edema in rats by various extracts from *Murraya koenigii* and *Ficus lacor* at 100mg/kg body weight

3.3.2 Anti-arthritis activity

3.3.2.1 Inhibition of adjuvant induced arthritis in rats (Administered daily from 15-21st day):

The separated extracts from the two plants were screened for their capacity to inhibit adjuvant induced poly arthritis in Rats at different doses given i.p. (Administered daily from days 15-21): The observed inhibitions are tabulated in tables.

3. 16: Adjuvant induced poly arthritis Inhibition in rats (Dose administered daily from 15-21st day) by various extracts from *Murraya koenigii*

Gro ups	Isolated Extracts	Dose mg/kg	Mean arthritic score± S.E.M. for days							
			15	16	17	18	19	20	21	22
I	Normal	1 ml i.p.	13.4± 1.3	13.8± 1.4	14.1± 1.3	14.8± 1.4	14.9± 1.5	15.0±1 .3	15.2±1 .4	15.2±1.5
II	MKPE	50 mg i.p.	13.1± 1.4	10.5± 1.3	10.3± 1.2	9.6± 1.2*	9.4± 1.0@	9.2± 0.6@	8.9±0. 6#	8.6± 0.7#
III	MKPE	100mg i.p.	13.2± 1.3	9.8± 1.1*	9.3± 1.0*	9.0± 1.2@	08.7± 1.0@	08.6± 0.9#	08.4± 0.8#	08.2± 0.9#
IV	MKEA	50mg i.p.	13.3± 1.2	13.4± 1.3	13.0± 1.1	13.1± 1.3	12.6± 1.0	12.4± 1.1	12.4±1 .0	12.5± 1.2
V	MKEA	100mg i.p.	13.3± 1.3	13.1± 1.2	12.8± 1.0	12.6± 1.3	12.5± 1.1	12.5± 1.0	12.5± 1.2	12.4± 1.1
VI	MKCF	50mg i.p.	13.1± 1.3	12.4± 1.2	11.6± 1.0	11.2± 1.1	10.5± 1.0*	9.9± 0.9*	9.2±0. 8@	8.9± 0.9#
VII	MKCF	100 mg i.p.	13.0± 1.4	11.5± 1.2	11.0± 1.0	10.1± 1.0	9.8± 1.0*	9.1± 0.9*	8.6±0. 8@	8.0± 0.7#
VIII	MKAf-1	100 mg i.p.	13.1± 1.3	11.2± 1.1	10.5± 1.0	10.2±1. 3	9.5±0. *.9	9.2±0. 9*	8.6±0. 8#	7.8±0. 70#
IX	Indomet hacin	2.5 mg i.p.	13.2± .2	9.8± 1.0*	9.3± 1.0*	9.0± 1.2*	8.7± 0.8#	7.6± 0.8@	07.0± 0.9@	6.4± 0.8#

Group I- Treated with saline Arthritic rats, Group II - Treated with fraction MKPE, 50mg/kg, Group III - Treated with fraction MKPE, 100 mg/kg, Group IV - Treated with fraction MKEA, 50 mg/kg, Group V - Treated with fraction MKEA, 100 mg/kg, Group VI - Treated with fraction MKCF, 50mg/kg, Group VII - Treated with fraction MKCF, 100mg/kg, Group VIII - Treated with fraction MKAf-1, 100mg/kg, Group IX- Treated with standard drug Indomethacin at the dose of 2.5mg/kg body weight, *p < 0.05, @p < 0.01, #P <0.001as compared with arthritic control.

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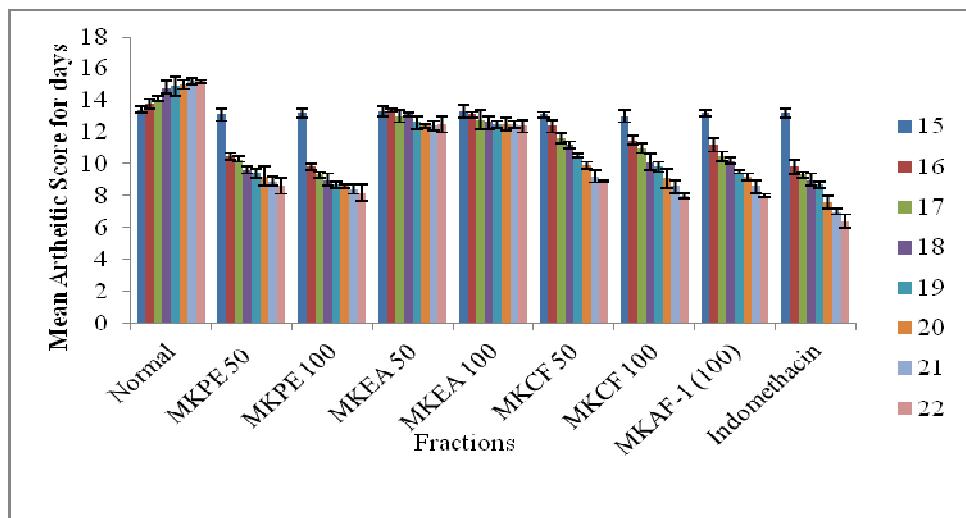


Figure 3.23: Inhibition of poly arthritis in rats induced via adjuvant (dose given daily from 15-21st day) by various fractions from roots of *Murraya koenigii*

3. 17: Inhibition of adjuvant induced poly arthritis in rats (Dose administered daily from days 15-21) by various extracts from *Ficus lacor* aerial roots

GR OU PS	Isolated extracts	Dose mg/kg	Mean arthritic score± S.E.M. for days							
			15	16	17	18	19	20	21	22
I	Normal	1 ml i.p.	13.4 ± 1.3	13.8 ± 1.4	14.1 ± 1.3	14.8 ± 1.4	14.9 ± 1.5	15.0 ± 1.3	15.2 ± 1.4	15.2 ± 1.5
II	FLPE	50 mg i.p.	13.0 ± 1.4	10.4 ± 1.4	10.0 ± 1.1	9.2 ± 1.0*	9.0 ± 1.0*	8.6 ± 0.9*	8.0 ± 0.8*	7.4 ± 0.8#
III	FLPE	100mg i.p.	13.3 ± 1.3	9.2 ± 1.1*	9.1 ± 1.0*	9 ± 1.1*	8.4 ± 0.9	8.0 ± 0.8@	7.4 ± 0.8#	7.0 ± 0.9#
IV	FLEA	50mg i.p.	13.0 ± 1.4	10.4 ± 1.4	10.0 ± 1.1	9.6 ± 1.0	9.6 ± 1.0*	9.2 ± 0.9*	9.2 ± 0.8	9.0 ± 0.8@
V	FLEA	100mg i.p.	13.3 ± 1.3	10 ± 1.3	9.6 ± 1.1	9.6 ± 1.0	9.6 ± 1.0*	9.0 ± 0.9*	9.0 ± 0.8	8.8 ± 0.8@
VI	FLCF	50 mg i.p.	13.0 ± 1.4	10.9 ± 1.4	10.8 ± 1.1	10.9 ± 1.0	10.7 ± 1.0	10.7 ± 0.9	10.6 ± 0.8*	10.6 ± 0.8*
VII	FLCF	100 mg i.p.	13.3 ± 1.3	10.8 ± 1.1	10.8 ± 1.0	10.8 ± 1.1	10.6 ± 0.9	10.6 ± 0.8	10.4 ± 0.8 *	10.4 ± 0.9 *
VIII	FLET	50 mg i.p.	13.0 ± 1.4	10.4 ± 1.4	10.0 ± 1.1	9.6 ± 1.0	9.2 ± 1.0*	8.6 ± 0.9*	8.0 ± 0.8	7.4 ± 0.8@
IX	FLET	100 mg i.p.	13.3 ± 1.3	10 ± 1.3	9.6 ± 1.1	9 ± 1.1*	8.4 ± 0.9*	8 ± 0.8@	7.0 ± 0.8 @	6.8 ± 0.2#
X	FLTP-1	100 mg i.p.	13.4 ± 1.3	12 ± 1.3	10.6 ± 1.1	10 ± 1.0	9.3 ± 0.9*	8.4 ± 0.8#	7.6 ± 0.7#	7.2 ± 0.7 @
XI	Indomth acin	2.5 mg i.p.	13.2 ± 1.2	9.8 ± 1.0*	9.3 ± 1.0*	9 ± 1.2*	8.7 ± 0.8#	7.6 ± 0.8@	7.0 ± 0.9 @	6.4 ± 0.8#

RESULTS AND DISCUSSION

Group I- Treated with saline Arthritic rats, Group II- Treated with fraction FLPE, 50mg/kg, Group III- Treated with fraction FLPE, 100 mg/kg, Group IV- Treated with fraction FLEA, 50 mg/kg, Group V- Treated with fraction FLEA, 100 mg/kg, Group VI- Treated with fraction FLCF, 50 mg/kg, Group VII- Treated with fraction FLCF, 100 mg/kg, Group VIII- Treated with fraction FLET, 50 mg/kg, Group IX- Treated with fraction FLET, 100 mg/kg, Group X- Treated with fraction FLTP-1, 100 mg/kg, Group XI- 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.

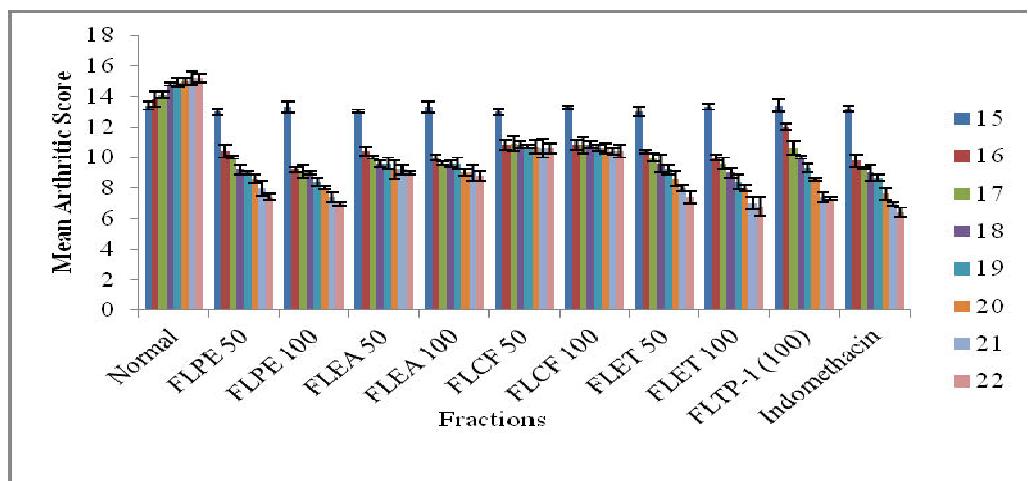


Figure 3.24: Inhibition of adjuvant induced poly rats (Given daily from 15-21st day) by various fractions from the aerial roots of *Ficus Lacor*

3.3.2.2 Effects of isolated extracts on body weight in adjuvant induced arthritic rats

3.18A: Effect of isolated extracts from *Murraya koenigii* and *Ficus lacor* on changes on body weight in adjuvant induced arthritic rats:

Groups	Isolated Extracts	Dose (mg/gm)	Before induction (gm)	After 7days (gm)	After 14days (gm)	On 21 st day (gm)
I	Normal Arthritic	1ml	160.85 ± 5.40	162.50 ± 5.20	140.40 ± 6.25	116.35 ± 5.65
II	MKPE	50	172.60 ± 4.50	168.42 ± 5.75	152.25 ± 5.50	145.75± 6.45*
III	MKEA	50	169.40 ± 3.60	165.25 ± 6.50	145.50 ± 7.30	122.20 ± 4.58
IV	MKCF	50	166. 30 ± 5.72	164.75 ± 5.50	158.50 ± 4.50	147.90 ± 5.25**
V	FLPE	50	182.50 ± 5.33	175.34 ± 5.56	161.45 ± 6.57	152.75 ± 5.90**
VI	FLEA	50	176.52 ± 4.75	164.45 ± 5.37	149.68 ± 5.64	138.45± 6.34
VII	FLCF	50	165.45± 5.68	153.50 ± 4.79	139.40 ± 6.75	127.50 ± 5.76*
VIII	FLET	50	174.50 ± 4.86	166.48 ± 5.50	155.50 ± 6.54	147.57 ± 4.48***
IX	Indomethacin	2.5	155.44 ± 7.50	149.79 ± 5.75	146.35 ± 5.50	142.90± 6.59***

RESULTS AND DISCUSSION

Group I- Edema Control (saline); 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.

3.18B: Effect of isolated extracts from *Murraya koenigii* and *Ficus lacor* on change in body weight in adjuvant induced arthritic rats:

Gro ups	Isolated Extracts	Dose (mg/gm)	Before induction (gm)	After 7days (gm)	After 14days (gm)	On 21 st day (gm)
I	Arthritic control	1ml	160.85 ± 5.40	162.50 ± 5.20	140.40 ± 6.25	116.35 ± 5.65
II	MKPE	100	165.50 ± 4.50	169.25 ± 4.50	156.29 ± 6.45	143.55 ± 3.45*
III	MKEA	100	172.58 ± 2.76	164.55 ± 4.50	154.24 ± 5.53	146.73 ± 5.36
IV	MKCF	100	171.30 ± 5.72	159.75 ± 5.50	162.50 ± 4.50	147.90 ± 5.25**
V	MKAf-1	100	175.30 ± 3.60	169.20 ± 4.50	152.50 ± 5.30	145.75 ± 3.65***
VI	FLPE	100	180.38 ± 4.68	167.34 ± 5.56	159.45 ± 6.57	152.75 ± 5.90***
VII	FLEA	100	174.32 ± 5.35	166.50 ± 4.74	140.58 ± 3.43	125.35 ± 4.39*
VIII	FLCF	100	169.38 ± 3.60	157.45 ± 4.79	145.56 ± 4.75	132.50 ± 5.60*
IX	FLET	100	173.60 ± 4.65	165.48 ± 5.50	159.50 ± 6.54	152.57 ± 4.48***
X	FLTP-1	100	180.50 ± 5.60	172.40 ± 4.20	165.30 ± 5.00	158.40 ± 4.25***
XI	Indome thacin	2.5	155.44 ± 7.50	149.79 ± 5.75	146.35 ± 5.50	142.90 ± 6.59***

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 FLET, Group X- Treated with fraction FLTP-1, isolated from *Ficus lacor*, Group XI- 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.

3.3.2.3 Effect on Organ weight in Adjuvant induced arthritic rats

3.19A: Effect of isolated extracts on Organ weight in Adjuvant induced arthritis in rats

Groups	Extracts	Dose mg/kg	Liver (gm)	Thymus (mg)	Spleen (mg)
I	Control arthritic	1ml	3.65 ± 0.26	87.45 ± 1.58	570.30 ± 2.95
II	MKPE	50	3.80 ± 0.39*	85.95 ± 2.25*	550.25 ± 3.40
III	MKEA	50	3.99 ± 0.50	88.36 ± 1.40	498.37 ± 2.50
IV	MKCF	50	4.10 ± 0.15**	94.75 ± 1.75	530.28 ± 4.64
V	FLPE	50	4.25 ± 0.26**	92.60 ± 2.24**	410.35 ± 2.64*
VI	FLEA	50	3.74 ± 0.19	83.50 ± 1.57	425.54 ± 2.45
VII	FLCF	50	3.99 ± 0.34*	90.35 ± 3.70**	558.80 ± 3.47
VIII	FLET	50	4.40 ± 0.20**	95.90 ± 1.50**	399.50 ± 2.89**
IX	Indomethacin	2.5	6.68± 0.18***	104.40 ± 1.68***	305.40 ± 2.26***

Group I- Edema Control (saline); 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.

3.19B: Effect of isolated extracts on Organ weight in Adjuvant induced arthritis in rats

Groups	Extracts	Dose mg/kg	Liver (gm)	Thymus (mg)	Spleen (mg)
I	Control arthritic	1ml	3.65 ± 0.26	87.45 ± 1.58	570.30 ± 2.95
II	MKPE	100	4.13 ± 0.24**	86.47 ± 1.25*	575.20 ± 2.24*
III	MKEA	100	3.60 ± 0.43	82.68 ± 2.45	569.29 ± 1.75*
IV	MKCF	100	5.45 ± 0.20***	98.65 ± 1.05**	366.46 ± 2.37**
V	MKAf-1	100	4.25.30**	95.40± 2.50**	460.20± 1.75***
VI	FLPE	100	5.39 ± 0.19**	95.48 ± 1.53	345.58 ±3.45***
VII	FLEA	100	3.48 ± 0.26	86.55 ± 2.75	530.30 ± 1.54
VIII	FLCF	100	4.02 ± 0.31*	90.35 ± 3.70**	489.45 ± 2.89
IX	FLET	100	5.63 ±0.13***	102.76±1.54** *	338.95 ±1.75***
X	FLTP-1	100	3.50±0.31**	93.60±2.40***	450.50±1.45***
XI	Indome thacin	2.5	6.68± 0.18***	105.40 ± 1.68***	305.40 ±2.26***

RESULTS AND DISCUSSION

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, isolated from *Murraya koenigii* roots, Group VI-Treated with fraction FLPE, Group VII- Treated with fraction FLEA, Group VIII-Treated with fraction FLCF, Group IX- Treated with fraction FLET, Group X-Treated with fraction FLTP-1, isolated from *Ficus lacor*, Group XI- 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.

3.3.2.4 Haematological parameters in adjuvant induced arthritic rats:

3.20A: Effect of isolated extracts from *Murraya koenigii* and *Ficus lacor* on haematological parameters in adjuvant induced arthritic rats:

Groups	Isolated Extracts	Dose (mg/gm)	Hb (gm%)	RBC count (million/cu.mm)	Total WBC count(cells/cu. mm)	ESR (mm/hr)
I	Arthritic Control	1ml	6.50±0.32	5.25 ± 0.10	11.25 ± 0.50	7.75± 0.25
II	MKPE	50	8.05±0.25**	6.10 ± 0.22**	10.20 ± 026**	7.10 ± 0.25*
III	MKEA	50	7.50 ±0.20	5.70 ±0.15	9.34 ± 0.30	6.95 ± 0.20
IV	MKCF	50	9.75±0.35**	6.20 ±0.20*	8.95 ± 0.23**	6.28 ± 0.32
V	FLPE	50	7.35 ±0.15**	6.40 ± 0.35***	9.16 ± 0.40*	6.15 ± 0.18
VI	FLEA	50	6.90 ±0.14*	6.32 ± 0.45	10.68 ± 0.35	7.30 ± 0.29
VII	FLCF	50	6.50 ±0.12	5.50 ± 0.33	10.75± 0.15	7.38 ± 0.30
VIII	FLET	50	9.15±0.30***	6.42 ± 0.28***	8.46 ± 0.28***	6.34 ± 0.40**
IX	Indomethacin	2.5	13.50±0.10** *	7.75 ±0.20***	6.75 ± 0.15***	3.75 ± 0.27***

Group I- Edema Control (saline); 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.

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3.20 B: Effect of isolated extracts from *Murraya koenigii* and *Ficus lacor* on haematological parameters in adjuvant induced arthritic rats:

Groups	Isolated Extracts	Dose (mg/g m)	Hb (gm%)	RBC count (million/cu.m m)	Total WBC count(cells/cu .mm)	ESR (mm/hr)
I	Arthritic control	1ml saline	6.50±0.32	5.6 ± 0.10	11.25 ± 0.50	7.75± 0.25
II	MKPE	100	8.65 ±0.36**	6.13 ±0.26**	9.80 ± 032*	6.90 ± 0.34**
III	MKEA	100	7.24 ±0.34	6.10 ±0.20	9.34 ± 0.19	6.84 ± 0.24
IV	MKCF	100	11.05 ±0.45***	6.48 ±0.25**	7.50 ± 0.35***	4.46 ± 0.32**
V	MKAf-1	100	11.80±0.50 ***	6.30±0.65***	7.40±0.40***	4.35±0.45***
VI	FLPE	100	9.85 ±0.20***	6.60 ± 0.42***	7.35 ± 0.24**	4.60 ± 0.15**
VII	FLEA	100	7.22 ±0.19	6.10 ± 0.37	8.10 ± 0.42	7.50 ± 0.25*
VIII	FLCF	100	7.50 ±0.32	6.30 ± 0.30	11.05± 0.15*	6.85 ± 0.43*
IX	FLET	100	12.59±0.26 ***	6.90 ± 0.27****	7.15 ± 0.28***	4.50 ± 0.40***
X	FLTP-1	100	9.80±0.55* **	6.70±0.37***	7.20±0.65***	4.90±0.28***
XI	Indome thacin	2.5	13.50±0.10 ***	7.75 ±0.20****	6.75± 0.15***	3.75±0.27***

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 FLET, Group X- Treated with fraction FLTP-1, isolated from *Ficus lacor*, Group I- 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.

3.3.2.5: Interleukin-1 (IL-1) and Tumor necrosis factor- α (TNF- α) determination in serum

3.21 A: Effect of various extracts from *Murrya Koenigii* and *Ficus lacor* on Tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) in serum of arthritic rats

Groups	Isolated Extracts	Dose mg/kg	IL-1 (ng/ml)	TNF- α (ng/ml)
I	Normal	1 ml i.p.	0.395 \pm 0.084	0.108 \pm 0.037
II	MKPE	50	0.698 \pm 0.176*	0.195 \pm 0.026*
III	MKEA	50	0.620 \pm 0.182	0.189 \pm 0.043
IV	MKCF	50	0.610 \pm 0.179*	0.180 \pm 0.033**
V	FLPE	50	0.575 \pm 0.105**	0.178 \pm 0.046**
VI	FLEA	50	0.695 \pm 0.125	0.193 \pm 0.024
VII	FLCF	50	0.720 \pm 0.156	0.188 \pm 0.029
VIII	FLET	50	0.595 \pm 0.135**	0.170 \pm 0.047**
IX	Indomethacin	2.5	0.475 \pm 0.142***	0.135 \pm 0.050***

Group I- Edema Control (saline); Group II- Treated with fraction MKPE; Group III- Treated with fraction MKEA; Group IV- Treated with fraction MKCF, isolated from *Murraja 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.

3.21 B: Effect of various extracts from *Murrya Koenigii* and *Ficus lacor* on interleukin-1 (IL-1) and Tumour necrosis factor- α (TNF- α) in serum in serum of arthritic rats

Groups	Extracts	Dose mg/kg	IL-1	TNF- α
I	Normal	1 ml i.p.	0.395 \pm 0.084	0.108 \pm 0.037
II	MKPE	100	0.680 \pm 0.158***	0.198 \pm 0.036**
III	MKEA	100	0.660 \pm 0.174*	0.157 \pm 0.045*
IV	MKCF	100	0.506 \pm 0.140**	0.163 \pm 0.058***
V	MKAf-1	100	0.490 \pm 0.165***	0.155 \pm 0.044***
VI	FLPE	100	0.510 \pm 0.145***	0.159 \pm 0.043***
VII	FLEA	100	0.745 \pm 0.165*	0.195 \pm 0.035*
VIII	FLCF	100	0.760 \pm 0.176*	0.186 \pm 0.044*
IX	FLET	100	0.495 \pm 0.127***	0.155 \pm 0.025***
X	FLTP-1	100	0.492 \pm 0.125***	0.148 \pm 0.045***
XI	Indomethacin	2.5	0.475 \pm 0.138***	0.135 \pm 0.050***

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 *Murraja koenigii*, Group VI- Treated with fraction FLPE, Group VII- Treated with fraction FLEA, Group VIII- Treated with fraction FLCF, Group IX- Treated with fraction FLET, Group X- Treated with fraction FLTP-1, isolated from *Ficus lacor*, Group XI- 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.

RESULTS AND DISCUSSION

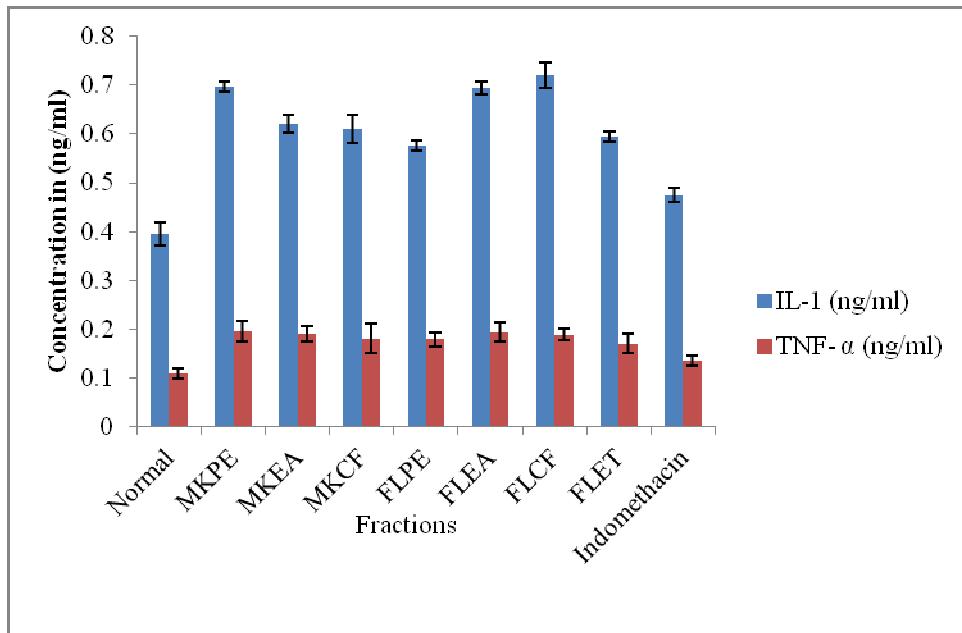


Figure 3.25: Effect of various extracts from *Murrya Koenigii* and *Ficus lacor* on interleukin-1 (IL-1) and Tumour necrosis factor- α (TNF- α) in adjuvant induced poly arthritis in rats serum at 50mg/kg body weight

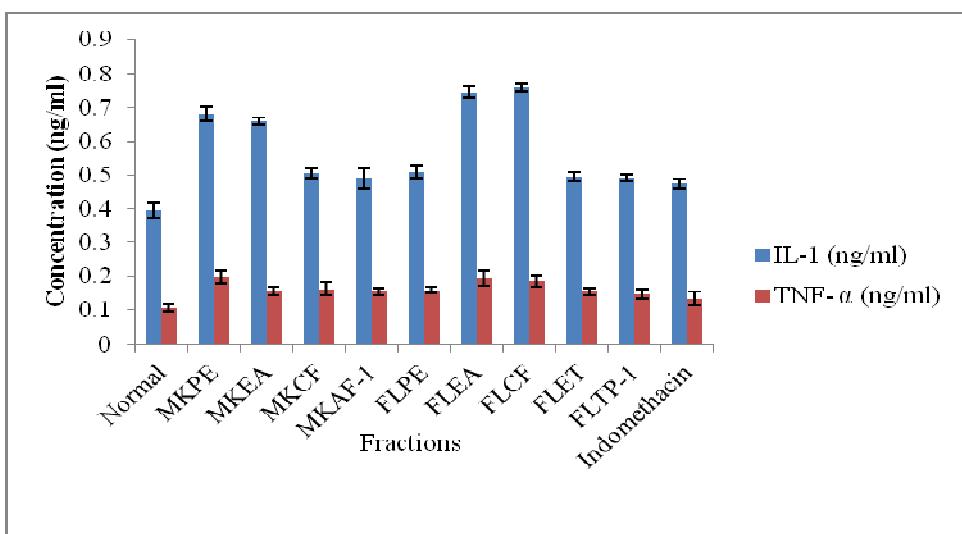


Figure 3.26: Effect of various extracts from *Murrya Koenigii* and *Ficus lacor* on interleukin-1 (IL-1) and Tumour necrosis factor- α (TNF- α) in serum of adjuvant induced poly arthritis in rats at 100mg/kg body weight

3.3.2.6 Inhibition of lysosomal enzyme activities in adjuvant induced polyarthritic rats

Inhibition of lysosomal enzyme activities in adjuvant induced polyarthritic rats (Administered daily from days 15-21):

The separated extracts from the two plants were screened for their capacity to inhibit lysosomal enzyme activities and urinary collagen and proteoglycan degradation products levels in adjuvant induced poly arthritic rats at different doses given intraperitonealy (Administered daily from days 15-21): The observed inhibitions are tabulated in Tables.

Table 3.22: Effect of various extracts from *Murraya koenigii* on lysosomal acid phosphatase activities in various tissues of adjuvant induced arthritic rats.

Groups	Isolated Extracts	(Acid phosphatase (β moles $\times 10^{-2}$ of Phenol)			
		Blood	Liver	Kidney	Spleen
I.	Normal control	0.15 ± 0.02	2.21 ± 0.21	1.35 ± 0.14	3.28 ± 0.15
II.	Arthritic Control	0.28 ± 0.02 ⁺	4.44 ± 0.29 ⁺	2.29 ± 0.17 ⁺	4.56 ± 0.39 ⁺
III.	MKPE	0.2± 0.02***	2.5± 0.24**	1.89 ± 0.18**	3.8 ± 0.25 **
IV.	MKEA	0.24± 0.012	3.27± 0.21	1.35 ± 0.11	3.31 ± 0.15
V.	MKCF	0.18 ± 0.02 ***	2.7 ± 0.26**	1.50 ± 0.15***	3.29 ± 0.22 *
VI.	MKAf-1	0.16± 0.01 ***	2.6± 0.22 ***	1.48± 0.26 ***	3.22± 0.18 ***
VII.	Indimethacin	0.14 ± 0.01***	2.42.33± 0.22***	1.40± 0.13 ***	3.01± 0.24 **

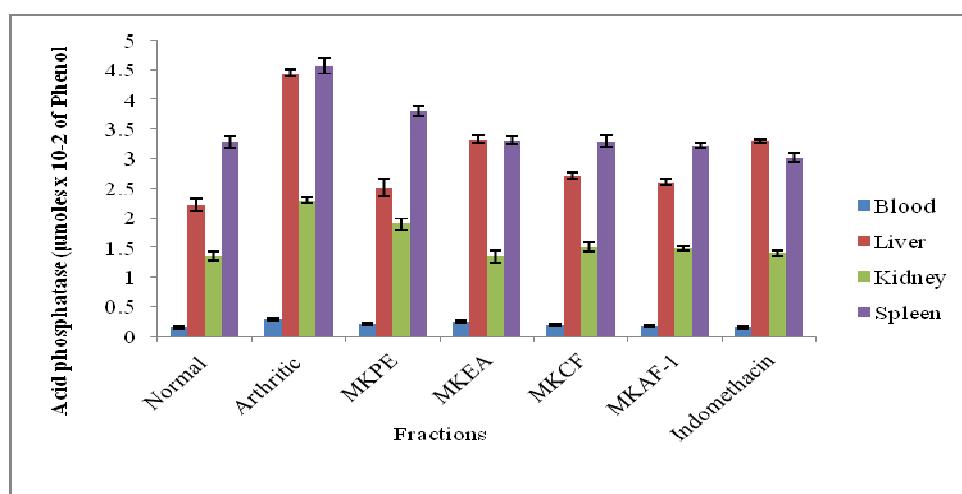


Figure 3.27: Effect of various extracts from *Murraya koenigii* on lysosomal acid phosphatase activities in various tissues of arthritic rats.

RESULTS AND DISCUSSION

Table 3.23: Effect of various extracts from *Ficus lacor* on lysosomal acid phosphatase activities in various tissues of arthritic rats

Groups	Isolated Extracts	acid phosphatase ($\mu\text{moles} \times 10^{-2}$ of Phenol)			
		Blood	Liver	Kidney	Spleen
I.	Normal control	0.15 ± 0.02	2.21 ± 0.21	1.35 ± 0.14	3.28 ± 0.15
II.	Arthritic Control	0.28 ± 0.02 + + + +	4.44 ± 0.29 + + + +	2.29 ± 0.17 + + + +	4.56 ± 0.39 + + + +
III.	FLPE	0.12 ± 0.02*** 0.02*** 0.02*** 0.02*** 0.02*** 0.02*** 0.02*** 0.02***	2.17 ± 0.26*** 0.26*** 0.26*** 0.26*** 0.26*** 0.26*** 0.26*** 0.26***	1.23 ± 0.15*** 0.15*** 0.15*** 0.15*** 0.15*** 0.15*** 0.15*** 0.15***	3.02 ± 0.22** ** ** ** ** ** ** **
IV.	FLEA	0.24 ± 0.02	3.33 ± 0.22	1.35 ± 0.12	3.31 ± 0.24
V.	FLCF	0.22 ± 0.02	2.47 ± 0.22	1.35 ± 0.13	3.31 ± 0.12
VI.	FLET	0.16 ± 0.02*** *** *** *** *** *** *** ***	2.7 ± 0.26* * * * * * * *	1.58 ± 0.15* 0.15* 0.15* 0.15* 0.15* 0.15* 0.15* 0.15*	3.38 ± 0.22** ** ** ** ** ** ** **
VII.	FLTP-1	0.15 ± 0.01*** 0.01*** 0.01*** 0.01*** 0.01*** 0.01*** 0.01*** 0.01***	2.5 ± 0.20*** 0.20*** 0.20*** 0.20*** 0.20*** 0.20*** 0.20*** 0.20***	1.50 ± 0.10*** 0.10*** 0.10*** 0.10*** 0.10*** 0.10*** 0.10*** 0.10***	3.25 ± 0.18** 0.18** 0.18** 0.18** 0.18** 0.18** 0.18** 0.18**
VIII.	Indomethacin	0.14 ± 0.01*** 0.01*** 0.01*** 0.01*** 0.01*** 0.01*** 0.01*** 0.01***	2.42 ± 0.22*** 0.22*** 0.22*** 0.22*** 0.22*** 0.22*** 0.22*** 0.22***	1.40 ± 0.13*** *** *** *** *** *** *** ***	3.01 ± 0.24** ** ** ** ** ** ** **

Group I- Normal control; Group II- Arthritic Control; Group III - Arthritic rats treated with FLPE, Group IV - Arthritic rats treated with FLEA, Group V - Arthritic rats treated with FLCF, Group VI - Arthritic rats treated with FLET from *Ficus lacor*, Group VII- Arthritic rats treated with Indomethacin 2.5mg/kg; Values represent MEV ± SEM; MEV (Mean Edema volume) and P.I. = Percentage inhibition, *p<0.05; **p<0.01; *** p <0.001, as compared with arthritic control.

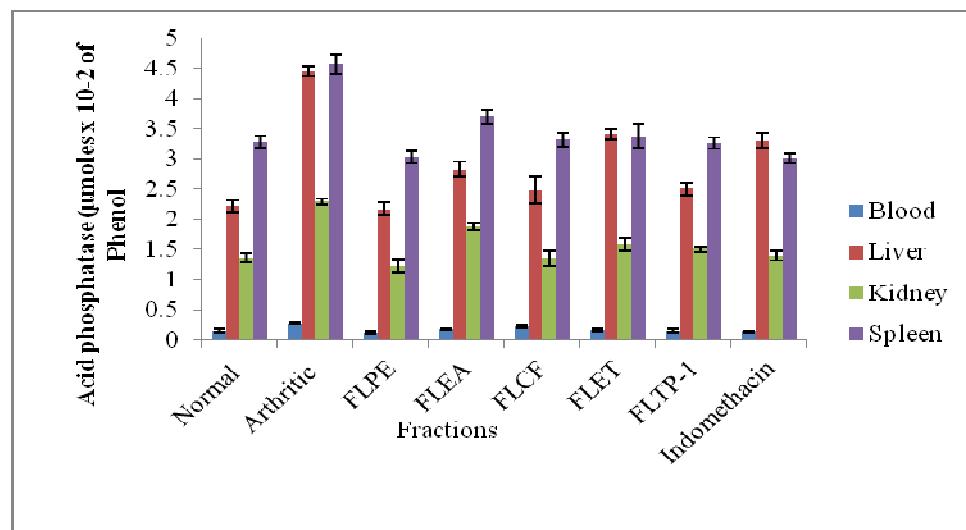


Figure 3.28: Effect of different extracts from aerial roots of *Ficus lacor* on lysosomal acid phosphatase activities in different tissues of arthritic rats

3.4 Antioxidant activity

3.4.1 Phenolic and Flavanoid contents of *Ficus lacor*

Phenolics compounds are the widely extended secondary plant metabolite in herbal drugs. The antioxidant potential of phenols is generally due to their hydrogen donor, redox properties, and oxygen singlet quenchers (Rice-Evans *et al.*, 1995). The total phenolic compounds were anticipated to be equivalent to 46.65 ± 1.75 mg of gallic acid. The flavonoids are a group of plant phenols with existing antioxidant potential (Pietta, 2000). The Total flavonoids contents were observed 38.42 ± 2.46 mg of catechin/gm of dried aerial roots extract, respectively.

3.4.2 Free radical scavenging activity of *Ficus lacor*

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) antioxidant evaluation is based on the capability of DPPH a established free radical, and decolorize in the subsistence of antioxidants. DPPH radical consist of an odd electron, which is accountable for the absorbance at 517nm. The antioxidant compound electron release and DPPH receive it. The DPPH is decolorized which be capable of quantitatively calculated from the changes in the absorbance. The percentage of free radical scavenge activity of alcohol extract and the ascorbic acid calculated at various concentrations (10; 20; 40; 80 and 160) and at upper concentration it was found to be 49.18percent and 62.24 percent for ascorbic acid as given in table 3.24. The absorbance was declining as the concentration of extract escalating as shown in table 3.24.

Table 3.24: Free radical scavenging activity of *Ficus lacor* ethanol extract

Sr. No	Concentration (μ g/ml)	Absorbance (517nm)		DPPH % inhibition	
		Ethanol Extract	Ascorbic acid	Ethanol Extract	Ascorbic acid
1.	10	0.540	0.503	11.48	17.38
2.	20	0.512	0.465	16.07	23.79
3.	40	0.480	0.405	21.32	33.60
4.	80	0.410	0.314	32.35	44.10
5.	160	0.308	0.225	49.18	62.24

* Values are expressed as mean \pm SEM of 3 observations

3.4.3 NO scavenging activity of *Ficus lacor*

The nitric oxide (NO) scavenging activity was evaluated at different concentrations (6.91%; 15.13%; 23.19%; 30.31 % and 52.26%) at extract concentrations of 100; 200; 400; 800 and 1000 μ g/ml, respectively). This is given in figure 29. The incubation of sodium nitroprusside solution in phosphate buffered saline at temperature of 25 °C for 150 min resulted nitrite production linear time-dependent, which was reduced in a concentration dependent manner by ethanol extract. The absorbance was decrease as concentration of the extract increased as shown in figure 3.30.

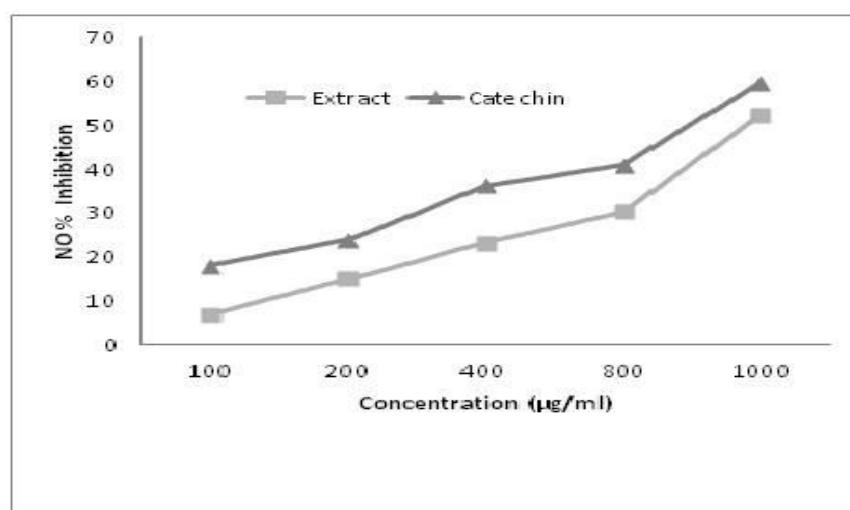


Figure 3.29: NO % scavenging activity of Ethanol extract of aerial roots of *Ficus lacor* in different concentrations ($\mu\text{g}/\text{ml}$) comparison with standard (Catechin), NO = Nitric oxide

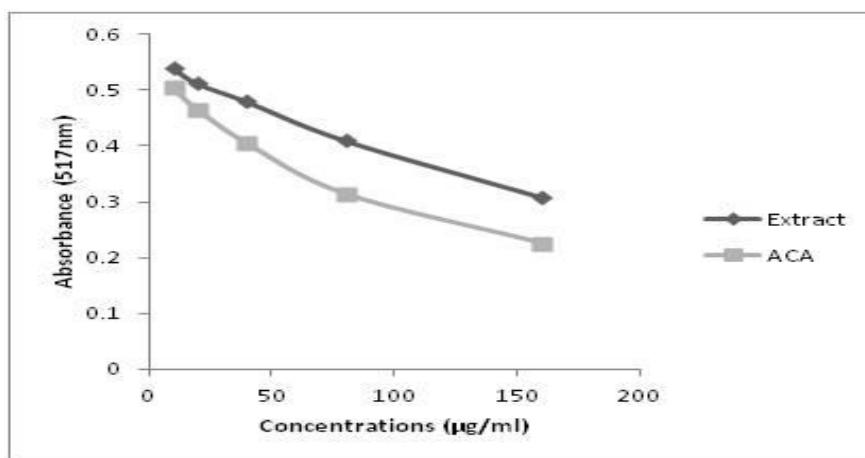


Figure 3.30: Absorbance of NO scavenging activity of ethanol extract isolated from aerial roots *Ficus lacor* at different concentrations in ($\mu\text{g}/\text{ml}$) compare with standard (Catechin); NO =Nitric oxide.

3.4.4 Phenolic and Flavanoid contents of *Murraya koenigii* roots

The Phenolic compounds are the widely spread secondary metabolite in the herbal drugs. Antioxidant potential of phenols are mostly due to their redox behavier, singlet oxygen quenchers and hydrogen donors (Rice-Evans *et al.*, 1995). The total phenolic compounds were expected to be equivalent to 51.23 ± 2.53 mg of gallic acid. The flavonoids are a group of plant phenolic compounds with general antioxidant property (Pietta, 2000). The total flavonoids contents were found to be 43.58 ± 1.89 mg of catechin/g of the dried root extract, respectively.

3.4.5 Free radical scavenging activity of *Murraya koenigii*

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) antioxidant evaluation is based on the capability of DPPH a established free radical, and decolorize in the subsistence of antioxidants. DPPH radical consist of an odd electron, which is accountable for the absorbance at 517nm. The antioxidant compound release an electron and DPPH receive it. The DPPH is decolorized which be capable of quantitatively calculated from the changes in the absorbance. The percentage of free radical scavenge activity of alcohol extract and the ascorbic acid calculated at various concentrations (10; 20; 40; 80 and 160) and at upper concentration it was found to be 54.92 percent and 62.24percent for ascorbic acid as given in table 3.25. The absorbance was decrease as the concentration of extract escalating as given in table 3.25.

Table 3.25: Free radical scavenging activity of *Murraya koenigii* ethanol extract

Sr. No	Concentratio n ($\mu\text{g/ml}$)	Absorbance (517nm)		DPPH % inhibition	
		Ethanol Extract	Ascorbic acid	Ethanol Extract	Ascorbic acid
1.	10	0.532	0.503	12.79	17.38
2.	20	0.520	0.465	14.75	23.79
3.	40	0.450	0.405	26.95	33.60
4.	80	0.380	0.314	37.35	44.10
5.	160	0.275	0.225	54.92	62.24

* Values are expressed as mean \pm SEM of 3 observations

3.4.6 NO scavenging activity

The nitric oxide (NO) scavenging activity was observed at different concentrations viz. 10.60%; 17.45%; 30.30%; 35.60% and 55.30%, at extract various concentrations (100; 200; 400; 800 and 1000 $\mu\text{g/ml}$). This is revealed in figure number 25. The incubation of sodium nitroprusside solution in phosphate buffered saline at 25 °C for 150 mins resulted, the nitrite production was reduced by ethanol extract in a manner of concentration-dependent. The absorbance was declined, as the concentration of the extract rising as show in figure number 3.31.

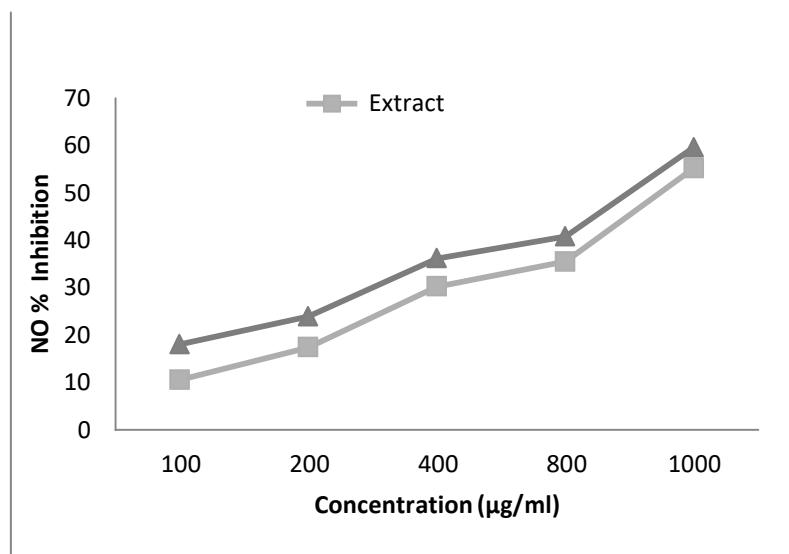


Figure 3.31: NO scavenging potential of extract isolated from *Murraya koenigii* roots at various concentrations ($\mu\text{g/ml}$) compared with standard (Catechin); NO= Nitric oxide.

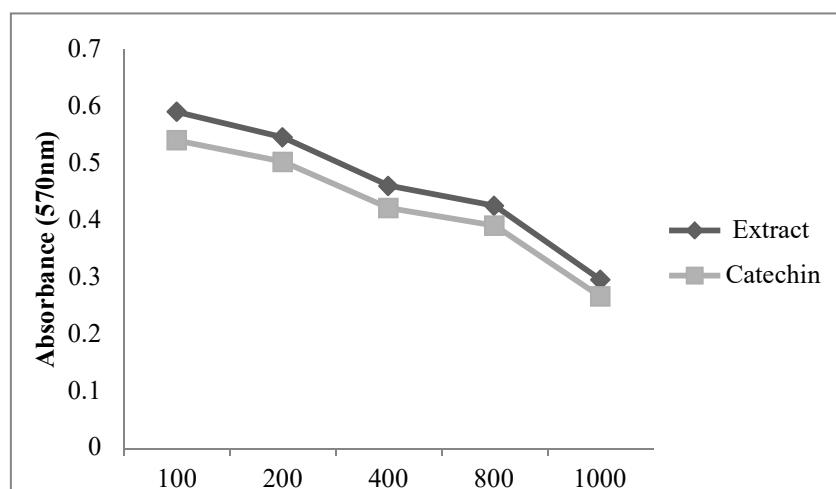


Figure 3.32: Absorbance of Nitric Oxide scavenging potential of Ethanol extract of roots *Murraya koenigii* at various conc. ($\mu\text{g/ml}$) compared with standard (Catechin).

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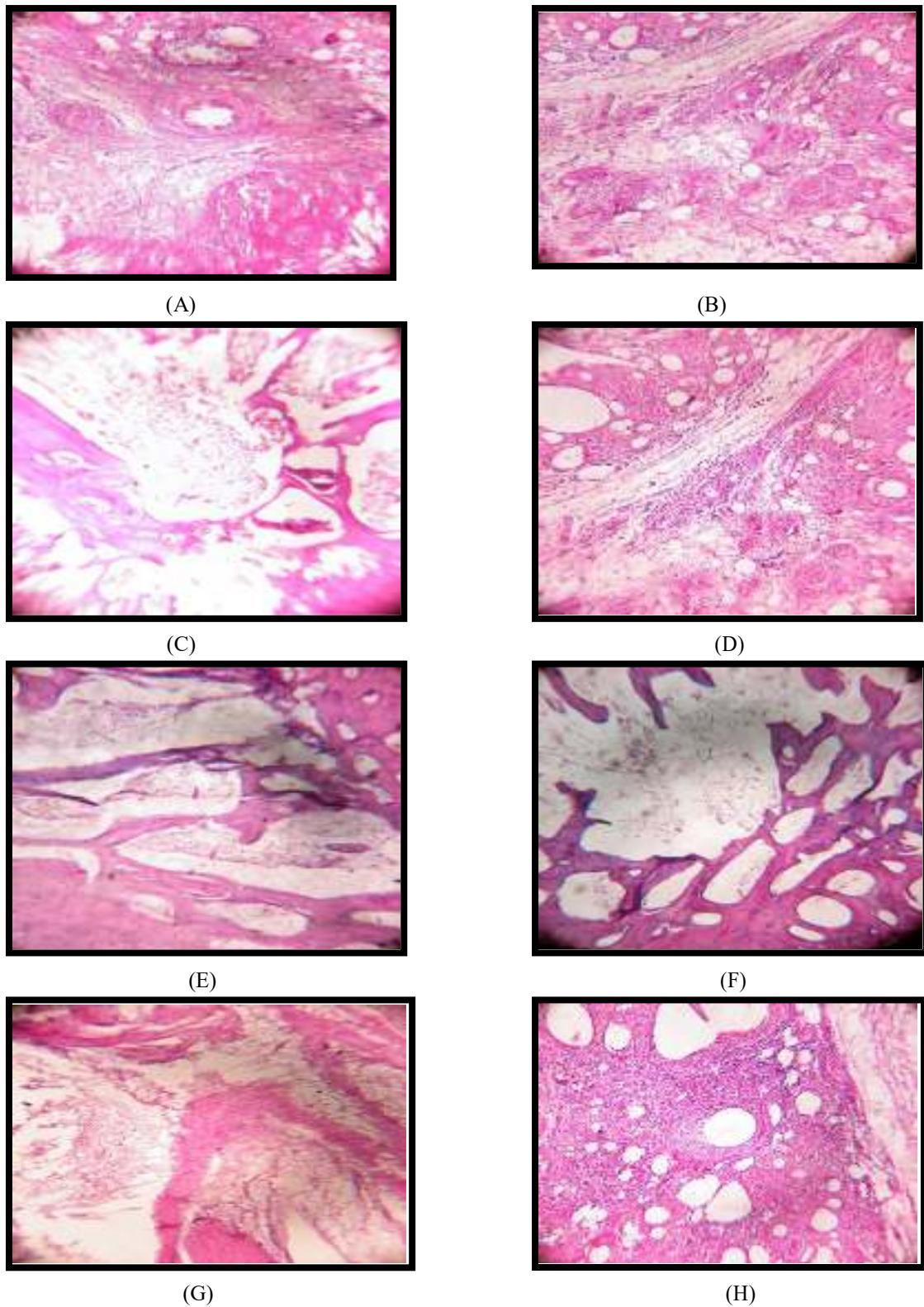


Figure 3.33: Histology of Adjuvant Induced Arthritic Rats Joints. A = Arthritic control, B = Treated with MKPE, C = Treated with MKCF, D = Treated with MKAF-1, E = Treated with FLPE, F = Treated with FLET, G = Treated with FLTP-1 and H = Treated with Indomethacin

3.5 Discussion

Carrageenan induced edema: The FLET fraction from *Ficus lacor* aerial roots showed maximum inhibition (75%) of Carrgeenan induced edema (Table 3.12B), 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.

It is reported that carrageenan induce the inflammation by escalating PE₂ release and leukocytes migration. It is moreover increase the expression of COX-2 in skeletal muscle, epidermis and inflammatory cells in air-pouch models, suggestive of that production of prostaglandin E₂ is connected through the expression of cyclooxygenase-2 (Sedgwick and Lees 1986) (Nantel et al 1999).

Inflammation induced via carrageenan involves three distinct phases of the discharge of the mediator; as well as serotonin & histamine in the primary phase (0- 2 h); kinins released in the second phase (3 h) and PG in the 3rd phase (>4 h) (Singh et al 1996). As mentioned earlier, FLET, FLPE and MKCF were effective in inhibition in all phases showing its inhibition of kinins as well as arachidonic acid. MKEA similarly but very less effective in all phases than FLET. The alkaloidal fraction (MKAF-1) and terpenoidal fractions (FLTP-1) showed significant inhibition as compared with standard drug. The anti-edematous response was also significantly decreased in rats pre-treated with indomethacin; the known COX inhibitor. MKPE showed prominent inhibition till the end of 2 hr and their effect gradually decreased showing that their effect was due to inhibition of histamine in the first phase as well as partly due to inhibition of kinins.

Histamine and Serotonin induced paw edema: *Murraya koenigii* roots: The MKCF fraction (60%, $p<0.01$) (Table 3.13B) shows greater inhibition of histamine induced edema as compared to MKPE and MKEA (Table 3.13B). MKEA shows slightly significant inhibition of Histamine induced edema (35.66%, $p<0.05$) and even for

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serotonin induced edema (31.32, p<0.05) (Table 3.14B). 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* (Table 3.13B) 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 (Table 3.14B). FLEA and FLCF fraction shows insignificant inhibition of both Histamine and Serotonin induced edema. As histamine plays an important role in the effector phase of delayed-type hypersensitivity (Yamada and Sugasawa 1996) known to be involved in many inflammation disorders such as hepatitis and arthritis (Napoli et al 1996) (Grom and Hirsch 2000), the significant activity shown by some fractions indicates potential to inhibit histamine induced phase in hypersensitivity responses associated with some diseases involving inflammation. The terpenoidal fractions (FLTP-1) showed significant inhibition (p<0.001) as compared with standard drug.

Formalin induced paw edema: *Murraya koenigii* roots: MKCF (45.84%, p<0.01) and MKPE were found to be significant in reducing edema (44.46%, p<0.01) similar to Diclofenac (Table 3.15B). Diclofenac produced more significant inhibition of formalin induced edema in the later stages of edema. MKEA was not significant.

Ficus lacor aerial roots: FLET fraction from *Ficus lacor* aerial roots was found to show inhibition (Table 3.15A and B) slightly less in significance (50.00%, p<0.01) as compared to Diclofenac but showed significant inhibition (p<0.01) of edema in early and intermediate phases. FLET showed inhibition more significant (55.57, p<0.001) and comparable to Diclofenac in the later phases. FLEA and FLCF did not show significant inhibition at any stage. The terpenoidal fractions (FLTP-1) showed significant inhibition (p<0.001) as compared with standard drug. Dose related increase in activity was seen but not in all cases. According to (Yuh-Fung et al 1995), acute inflammation induced by formalin results cell damage, which involves in the production of endogenous mediators, like histamine; serotonin; bradykinin and prostaglandins. It is well inhibition of edema induced by formalin in rats is the most preferable test experiment to evaluate anti-arthritis and anti-inflammatory agents, as it closely resembles human arthritis (Greenwald 1991; Banerjee et al 2000). As some of the above fractions significantly inhibited this model of inflammation, they can assume that possess anti-inflammatory and anti-arthritis potential same as to diclofenac, a COX inhibitor.

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Adjuvant Induced polyarthritis: *Murraya koenigii* roots: MKPE and MKCF showed statistically significant reticence of arthritis lesions ($p<0.05$) from day 16, ($p<0.01$) from day 20 and ($p<0.001$) from 21st day onwards. The fractions were given in higher doses decreased the lesions to a more extent, showing a dose reliant reduction in lesions (Table 3.16). The extracts MKPE and MKCF showed significant increase in body weight ($p<0.001$) as compared to arthritic control group (Table 3.18 A and B) and increase in liver weight ($p<0.01$), increase in thymus weight ($p<0.001$) and decrease in spleen weight ($p<0.001$) in arthritis control. MKEA did not showed any significant result in organ weight and body weight estimation (Table 3.19 A and B). The extracts MKPE and MKCF showed significantly ($p<0.001$) decreased level of acid phosphatase in blood, whereas they also significantly ($p<0.001$) decreased the level of acid phosphatase in liver and kidney. MKEA was not able to produce a significant effect (Table 3.20). The extracts MKPE and MKCF showed significant decrease in WBC count ($p<0.001$); hemoglobin contents increased and counts of RBC as compared to control group (Table 3.20 A & B). There was significant difference between model group and control group in TNF- α and IL-1 level. After treatment with MKPE, MKEA and MKCF, the level of IL-1 and TNF- α in serum was lower than model group in MKPE and MKCF extracts (Table 3.21 A and B). Our study showed that MKPE and MKCF have a potent inhibitory effect on levels of TNF- α and IL-1. TNF- α induced synovial cell to make collagenase, especially type-2 collagenase to destroy the cartilage matrix, stimulate the synovial cell make prostaglandin E2 (PGE2) and support neutrophil adhere to endotheliocyte to promote synovium inflammatory reaction (Dayer *et al.*, 1985; Gamble *et al.*, 1985; Abramson and Amin, 2002). Brennan *et al.* found that if TNF- α was blocked-up using specific antibodies, IL-1 production ceased (Brennan *et al.*, 1989). IL-1 caused synonitis by stimulating heterophil granulocyte and promoting the metabolism of arachidonic acid, and destroyed the synovial cell by promoting collagenase and PGE2, and produced synovial fibroblast and cartilage cell (Mino *et al.*, 1998). These pro-inflammatory cytokines caused synovitis and hyperplasy appearance, and the synovitiswas related to synovial hyperplasia and disturbance apoptosis (Soden *et al.*, 1991). The alkaloidal fraction (MKAF-1) showed significant inhibition ($p<0.001$) as compared with standard drug. In the histological studies, the joints showed the destruction of swollen joints and continual migration into the synovium and joint fluid of polymorphonuclear leukocytes, lymphocytes and monocytes/macrophages, all of which generate inflammatory cytokines. Pharmacological inhibition of leukocyte migration and gathering in arthritis may have

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beneficial effects for joint preservation. In this study, histological interpretation supported that the administration of MKPE, MKCF (dose 100mg/kg body weight) may be protective by decreasing the leukocytic migration as shown in figure 3.33. The alcohol extract of roots has shown very significant total phenolic content, total flavonoids content and DPPH free radical scavenging activity (Table 3.25) and NO scavenging activity.

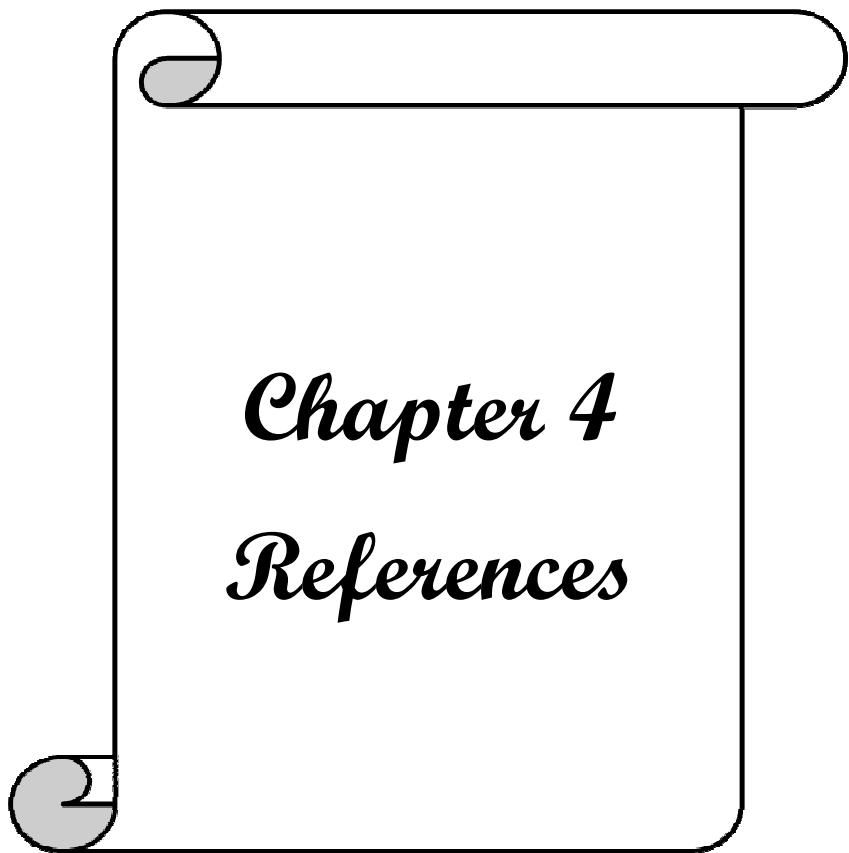
Ficus lacor aerial roots: FLPE and FLET were found to be significant in reducing edema as similar to standard drug. Standard drugs produced more significant inhibition of edema. FLPE and FLET showed statistically significant arthritic lesions inhibition ($p<0.05$) from day 16, ($p<0.01$) from day 20 and ($p<0.001$) from day 21 onwards. The extracts given at higher doses, decreased the lesions to a higher extent and it was showing a dose dependent decrease in lesions (Table 3.17). The fractions FLPE and FLET showing a significant raise in body weight ($p<0.001$) as compared to arthritic control group (Table 3.18 A and B) and increase in liver weight ($p<0.01$), decrease in spleen weight ($p<0.001$) and increase in thymus weight ($p<0.001$) in arthritis control. The fraction FLEA shownen insignificant result in organ and body weight estimation (Table 3.21 A and B). Fractions FLPE and FLET showing significant reduction in WBC count ($p<0.001$), augmented hemoglobin contents and counts of RBC as compared with control group (Table 3.20 A and B). The extracts FLPE and FLET showed significantly ($p<0.001$) decreased the level of acid phosphatase in blood, whereas they also significantly ($p<0.001$) decreased the level of acid phosphatase in liver and kidney. The fractions FLEA and FLCF did not show significant effect (Table 3.23). Here was significant differentiation between model group and control group in TNF- α and IL-1 level. Subsequent to treatment with various fractions FLPE, FLCF, FLEA & FLET the level of TNF- α and IL-1 in serum was lower than the model group in FLPE and FLET fractions (Table 3.21 A and B). Our study showed that FLPE and FLET fractions have a potent inhibitory effect on levels of TNF- α and IL-1. TNF- α induced synovial cell to compose collagens, especially type-II collagenase to destroy the cartilage matrix, stimulate the synovial cell compose prostaglandin E2 (PGE2) and promote neutrophil adhere to endotheliocyte to promote synovium inflammatory reaction (Dayer *et al.*, 1985; Gamble *et al.*, 1985; Abramson and Amin, 2002). Brennan *et al.* found that if TNF- α was blocked using specific antibodies, IL-1 production ceased (Brennan *et al.*, 1989). IL-1 caused synonitis by stimulating heterophil granulocyte and promoting the metabolism of arachidonic acid, and destroyed the synovial cell by promoting collagenase and PGE2, and produced synovial fibroblast and cartilage cell (Mino *et al.*, 1998). These cytokines

caused synovitis and hyperplasy appearance, and the synovitiswas related to synovial hyperplasia and disturbance apoptosis (Soden *et al.*, 1991).

In the histological studies, the joints showed the inflamed joints destruction and continued movement into the synovium and joint fluid of polymorphonuclear leukocytes, lymphocytes and monocytes, all of which produce inflammatory mediator's cytokines. Thus, pharmacological inhibition of this leukocyte migration and accumulation in arthritis may have beneficial effects for joint preservation. The histological interpretation supported that the administration of FLPE, FLET and FLTP-1 at higher dose may be protective by decreasing the leukocytic migration as shown in figure 3.33. The alcohol extract of *Ficus lacor* aerial roots have shown significant total phenolic content, total flavonoids content and DPPH free radical scavenger activity (Table 3.24) and NO scavenging activity.

3.6 Summary and Conclusion

In present scenario the use of complementary and alternative remedy is greater than ever and it offers unique opportunities for the development of natural medicine. Traditional knowledge of the past and present folk is of massive value to the development of newer drug compounds. Earlier studies are contributing so much in the isolation of the compounds which are responsible for the mechanism of action and therapeutic values in various types of ailments. Many allopathic medicines of daily use have been isolated from the natural sources. Now a day's inflammation, arthritis and related diseases are increasing due to the changes in the current lifestyle. 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. 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. So therapeutic effect of *Murraya koenigii* roots and *Ficus lacor* aerial roots on inflammation and arthritis is first time reported.



Chapter 4

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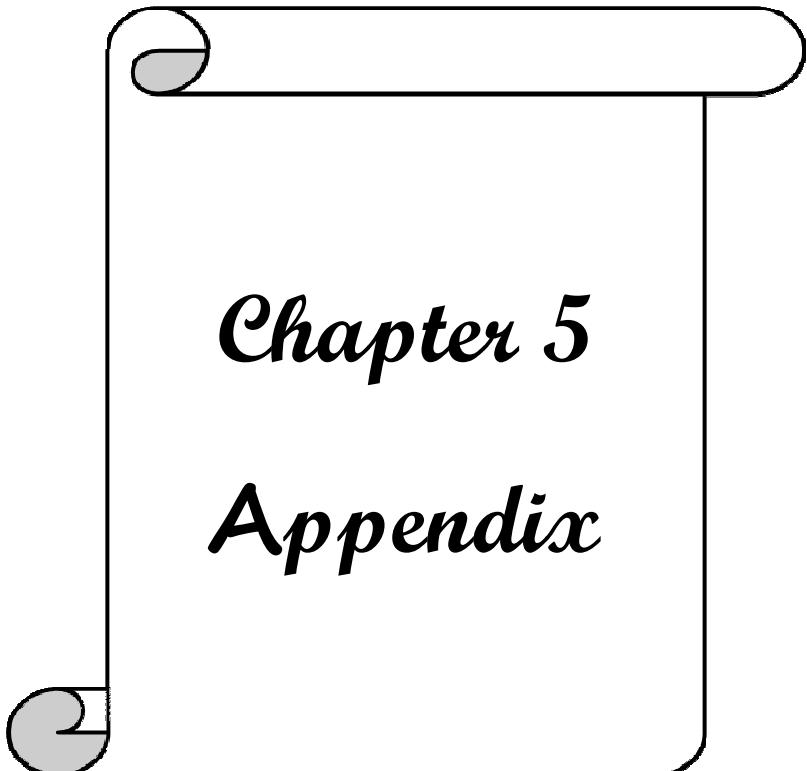
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Chapter 5

Appendix

Research Article

FREE RADICAL SCAVENGING AND ANTIOXIDANT POTENTIAL OF FICUS LACOR BUCH. HUM.

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ABSTRACT

Objectives: The objective of the present study was to evaluate the antioxidant potential of ethanol extract from aerial roots of *Ficus lacor*.

Methods: Ethanol extract of aerial roots of *Ficus lacor* was used to study their total phenolic and lavonoids contents, in vitro antioxidant including radical scavenging of T,T-Diphenyl- \cdot -picrylhydrazyl DPPC and nitric oxide NO.

Results: The ethanol extract showed significant results Total phenolic contents were estimated to be equivalents to 1.1 ± 0.1 mg of gallic acid equivalent and total lavonoids contents were 1.1 ± 0.1 mg of catechin/g equivalent. The percentage of DPPC and NO scavenging activity increased with increasing various concentration of extract.

Conclusions: The results concluded that the extract have potential source of antioxidants of natural origin that could have great importance as therapeutic agents for biological system liable to free radical mediated reactions.

Keywords: Antioxidant, *Ficus lacor*, DPPC, NO, phenolic content, lavonoids content

INTRODUCTION

Plants are used as medicine around the world and plant based medicine has been the stronghold of traditional societies in dealing with health problems [1]. The World Health Organization has estimated that 80% of the population rely upon traditional medicine for their primary health care needs [2, 3]. The overall, medicinal plants are the backbone of the traditional medicine. Plants are a natural source of biologically active compounds known as phytoconstituents [1, 4]. The phytoconstituents have been found to act as antioxidants by scavenging free radicals, and many have therapeutic potential for free radical associated diseases. Reactive oxygen species ROS like hydrogen peroxide, superoxide anions, hydroxyl radicals, nitric oxide and peroxy nitrite radicals, play an important role in oxidative stress related to the pathogenesis of various types of diseases [5]. In healthy individuals, the production of free radicals is balanced by the antioxidative defence system of body. The oxidation of lipids, DNA, proteins, carbohydrates, and other biological molecules by toxic ROS may cause DNA mutation and serve to damage target cells or tissues, and this often results in cell senescence and death [6]. Biological antioxidants are natural molecules, which can prevent the uncontrolled formation of free radicals and activated oxygen species [7]. Biological antioxidants include anti-oxidative enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and small non-enzymatic antioxidant molecules, such as glutathione and vitamins C and E [8]. In recent years there has been an increasing interest in finding natural antioxidants since they can protect the human body from oxidative damage [9]. In the prevention of overproduction of free radicals in biological systems, the reinforcement of endogenous antioxidants via intake of dietary antioxidants mainly from plant sources may be of particular importance in decreasing the cumulative effects of the oxidatively damaged molecules [10].

The traditionally used medicinal plants look forward to such standardization and the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their effective pharmacological activities, low toxicity and economic viability [11]. Some of these plants have shown potent antioxidant activity [12]. However, majority of plants have not yet been evaluated for such activity. So, in order to contribute further the knowledge of Indian traditional medicinal plants, our present study is focussed on anti-oxidant activity of ethanol extract isolated form aerial roots of *Ficus lacor*. It is also known as *Ficus infectoria* Roxb. It is locally known as

pilkhan. It is widely distributed in tropical and subtropical regions of the world. It also grows in various humid regions in India. The bark of the plant in traditional system of India is used for treatment of ulcers, for expelling round worms, and for treatment of leucorrhoea. The leaves are also used for treatment of various skin problems [13]. This is first ever antioxidant and free radical scavenging activity on aerial roots of *Ficus lacor*.

Materials and Methods

Plant Collection

The plant of *Ficus lacor* aerial roots were collected during the month of July 2012 from Panchkula Sector-10, Chandigarh, North India. The plant material was taxonomically identified and authenticated by Dr. C.B. Singh, Head, Raw materials Herbarium and Museum division, with ref.no. N)SCA)R/R(MD/Consult/TTT-TT/T/1/1. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of Pharmacy, Chitkara University, Panjab for further reference.

Preparation of Extracts

The dark brown coarse powder 100g was extracted with ethanol in soxhlet apparatus for 4 h. The dark brown mass of extract 10g was obtained by concentrating ethanol extract in rotary vacuum evaporator.

Preliminary Phytochemical Studies

The Ethanol extract screened for preliminary phytochemical studies [14]. The presence of lavonoids, saponins, phenolic compounds, and sterol in extract were observed.

METHODS FOR SCREENING OF ANTIOXIDANT ACTIVITY:

Total phenolic content

Total phenolic content in the extract was determined using the Folin-Ciocalteu's reagent (FCR) according to Molan et al. [14]. Each



Evaluation of Phenolic contents and Antioxidant potential of *Murraya Koenigii (L) spreng roots*

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Key words: *Murraya koenigii*, Antioxidant, phenolic content, flavonoid content, DPPH, NO.

ABSTRACT

The present study was to evaluate the antioxidant potential of ethanol extract from roots of *Murraya koenigii*. Ethanol extract of roots was used to study their total phenolic and flavonoid contents and antioxidant including radical scavenging of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide. The ethanol extract showed significant results, Total phenolic contents were estimated to be equivalents to 51.23 ± 2.53 mg of gallic acid equivalent and total flavonoid contents were 43.58 ± 1.89 mg of catechin/g equivalent. The percentage of DPPH and NO scavenging activity increased with increasing various concentration of extract.

INTRODUCTION

Murraya koenigii Linn (Rutaceae) commonly known as Curry patta and Meethi neem, is an aromatic more or less deciduous shrub or a small tree up to 6 m in height found throughout India up to an altitude of 1500 meters. In traditional system of Medicine, it is used as antiemetic, antidiarrhoeal, dysentery, febrifuge, blood purifier, tonic, stomachic, flavouring agent in curries and chutneys (Anonymous, 1998; Prajapati *et al.* 2003).

The plant based medicine has been the stronghold of traditional societies in dealing with health problems (Fransworth, 1994). The World Health Organization has estimated that 80% of the population rely upon traditional medicine for their primary health care needs (Sindhu and Arora, 2012; Kurian, 1995). Plants are a natural source of biologically active compounds known as phytoconstituents (Fransworth, 1994). The phytoconstituents have been found to act as antioxidants by scavenging free radicals, and many have therapeutic potential for free radical associated diseases. Reactive oxygen species (ROS) including hydroxyl radicals, singlet oxygen, hydrogen peroxide and superoxide radicals are frequently generated as by products of biological reaction (Kikuzaki and Nakatani 1993).

However, these ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as Arthritis, carcinogenesis, DNA damage and various degenerative disorders such as neuro-degenerative diseases, aging and cardiovascular ailments etc. (Osawa, 1994; Noda *et al.* 1997).

MATERIALS AND METHODS

Plant Collection

The plant of *Murraya koenigii* roots were collected during the month of the July 2009 from Chitkara University (Punjab), North India. The plant material was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum division, with ref. no. NISCAIR/RHMD/ Consult/2010-11/1638/236. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of pharmacy, Chitkara university, Panjab for further reference.

Preparation of Extracts

The dark brown coarse powder 800g was extracted with ethanol in soxhlet apparatus 72 h. The dark brown mass of extract (35.75g) was obtained by concentrating ethanol extract in rotary vacuum evaporator.

* Corresponding Author

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ANTI-INFLAMMATORY POTENTIAL OF DIFFERENT EXTRACTS ISOLATED FROM THE

The present study was undertaken to evaluate the anti-inflammatory potential of mg/kg body weight (b.w.) using animal models of acute inflammation (carrageenan-, histamine- and serotonin-induced inflammation). The results of the inhibition, respectively, in the carrageenan-induced inflammation model. In histamine-induced inflammation, the M_T showed 69.10% and 68.72% inhibition in serotonin-induced inflammation.

Anti-inflammatory;



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Introduction

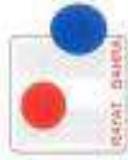
India has a rich cultural heritage of traditional medicine which chiefly comprised the two widely flourishing system i.e. Ayurvedic Siddha, Unani systems etc. and since The plant crude drugs are available easily in abundance comparatively cheaper. They have negligible side effects frequently prescribed to patients of all age group for therapeutic action and uses of these drugs are well described in classical literature on indigenous medicines in many plant books and pharmacopoeias[1,2]. Ficus genus contains over 800 species and about 40 genera of the genus Ficus. Ficus genus also known as fig genus. All Ficus species contain latex-like material within their vasculatures, affording self-healing from physical assaults[3]. Ficus auriculata, Ficus benghalensis (Indian banyan), Ficus carica, Ficus deltoidea, Ficus elastica (India rubber tree), Ficus lacor (pakur tree), Ficus lingua, Ficus macrophylla, Ficus microcarpa (Chinese banyan), Ficus pseudopalma, Ficus pumila, Ficus religiosa, Ficus



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Dr./ Mr./ Ms. RAKESH K. SWHU
Participated In the Deliberations of the Conference
as a Delegate and / presented a Poster.*


Prof. A.C. Rana
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Prof. S.C. Bedi
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Govt. of India



CERTIFICATE

Certified that Prof./Dr./Mr./Ms./.....Rakesh Sindhukumar.....of.....Chitkara University.....

participated/chaired the session/delivered invited talk/presented paper(oral/poster) in Conference organized by M.M. College of Pharmacy, Maharsi Markandeshwar University, Mullana-Ambala, (Haryana). Haryana Medical council accord credit hour of 10 hours & 30 min to the participants.

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IPS President

Prof. R.K. Goel
Executive member, IPS

Prof. Vipin Saini
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Prof. Sunmeet Gupta
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CHITKARA COLLEGE OF PHARMACY

"Advances in Pharmacological & Pharmaceutical Approaches to Drug Discovery & Clinical Development" (APPADDCD-2013)

This is to certify that Dr/ Mr/ Ms Rakesh Kumar Sardana of Chitkara University

participated in the International Conference "APPADDCD-2013" on 15th - 16th March, 2013 as student / Faculty / Industry

Participant. He/ she presented an Oral Presentation / Poster on Pharmacognostical Evaluation of Aerial Roots of Ficus daur.

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८, उ. ए. भवान, नई दिल्ली - ११००१२

Dr. K. S. KRISHNAN MARG, (Near Pusa Gate), NEW DELHI 110 012 6

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१०२-२५३८२६४५३०५, १०२-२५३८२६४५३०६

Ref. NISCAIR/RHMD/Consult/-2010-11/1638/236

January 11, 2011

Dr. H.B. Singh

Head

Raw Materials Herbarium & Museum (RHMD)

Phone: 011-25841143

E-mail: hbs@niscair.res.in; hbsbhati@yahoo.com

Dear Mr. Sindhu

Kindly refer to your letter No nil dated nil for identification of two herbarium specimens. The specimens have been identified as given below.

Sl No	Sample Received as	Part	Sample Identified as	Remarks
01	Ficus infectoria Roxb.	Aerial part	<i>Ficus locor</i> Buch.-Ham. Syn. <i>Ficus infectoria</i> Roxb.	
02	Murraya koenigii	Aerial part	<i>Murraya koenigii</i> (L.) Spreng.	

With regards

Yours sincerely

(Dr. H.B. Singh)

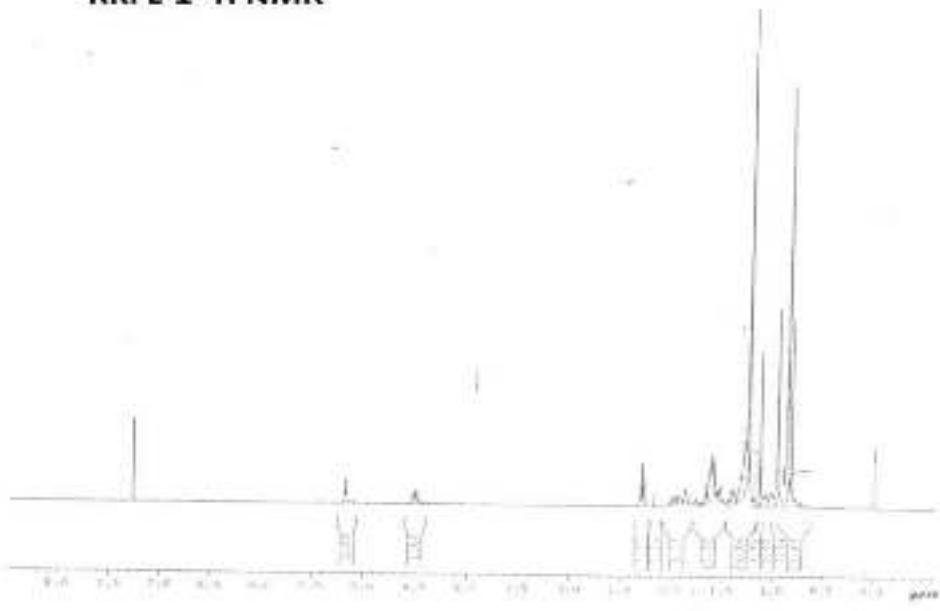
Mr. Rakesh Kumar Sindhu

C/O Mr. Inderbir Singh

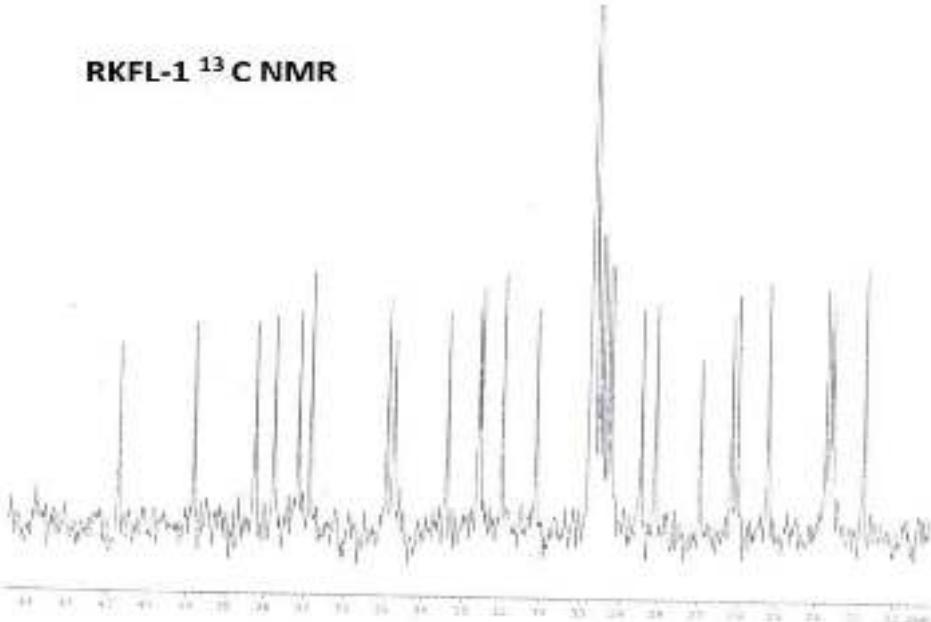
B-40, DLF Colony

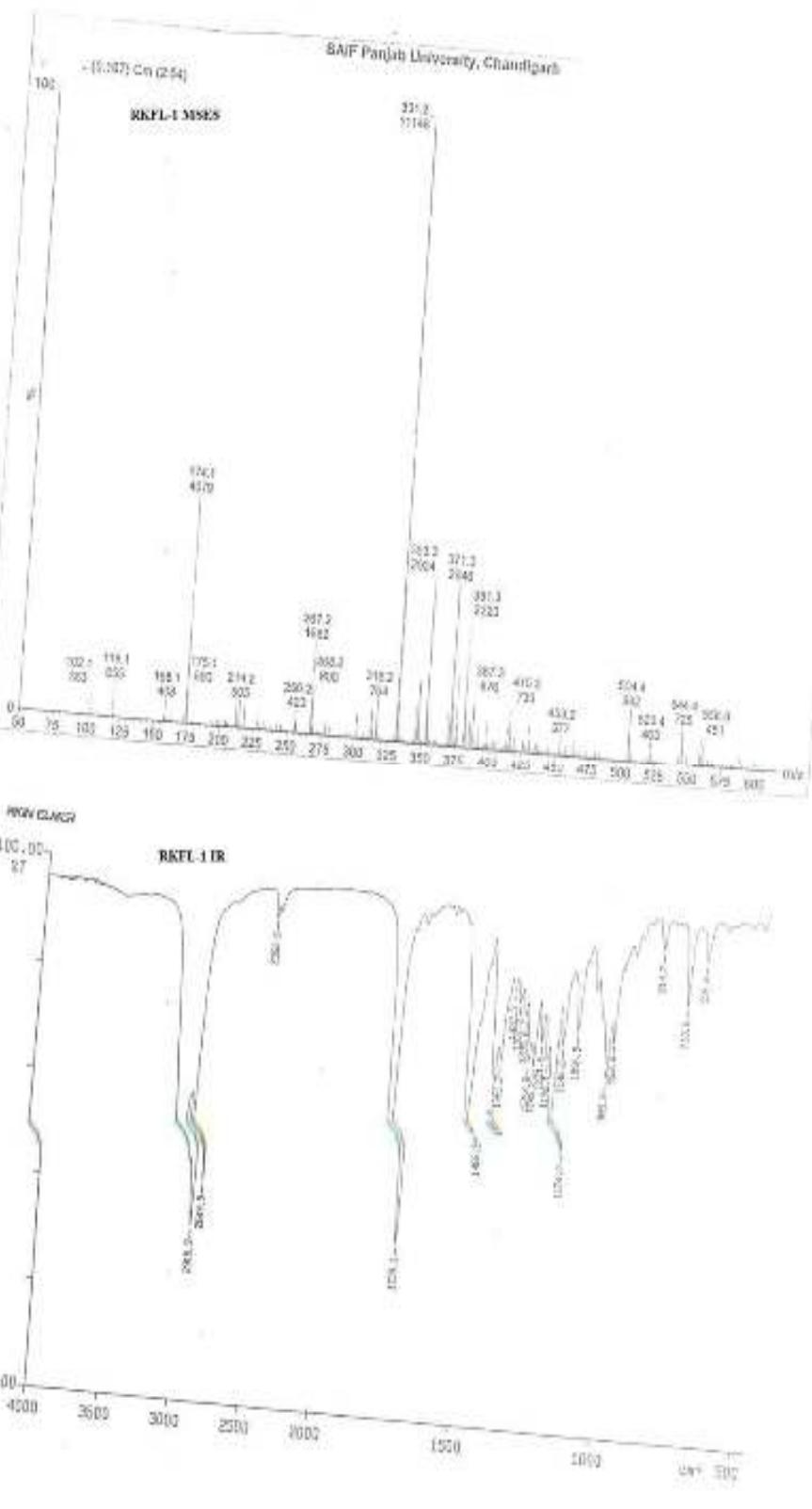
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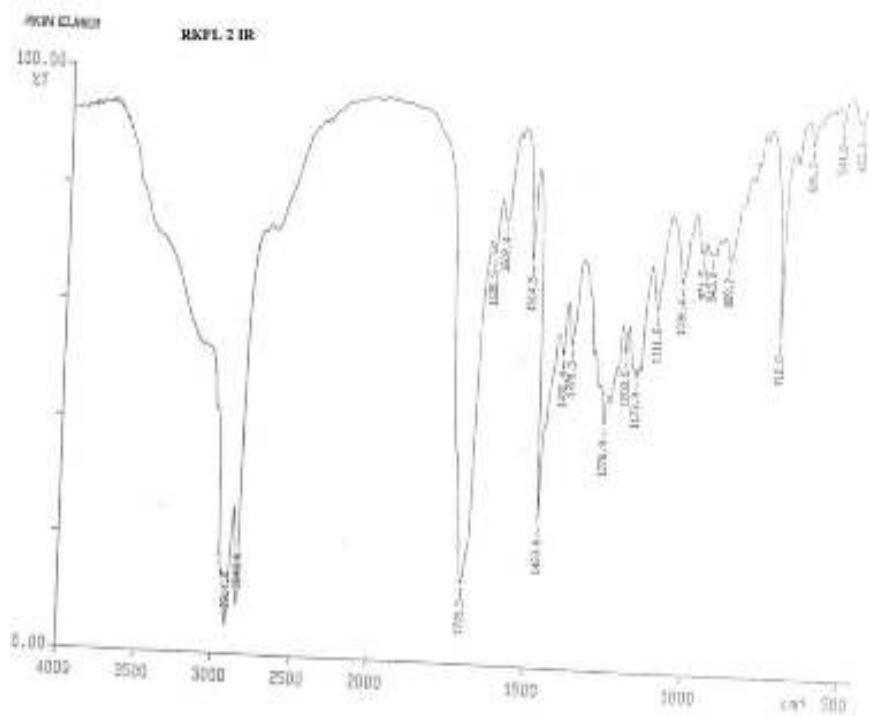
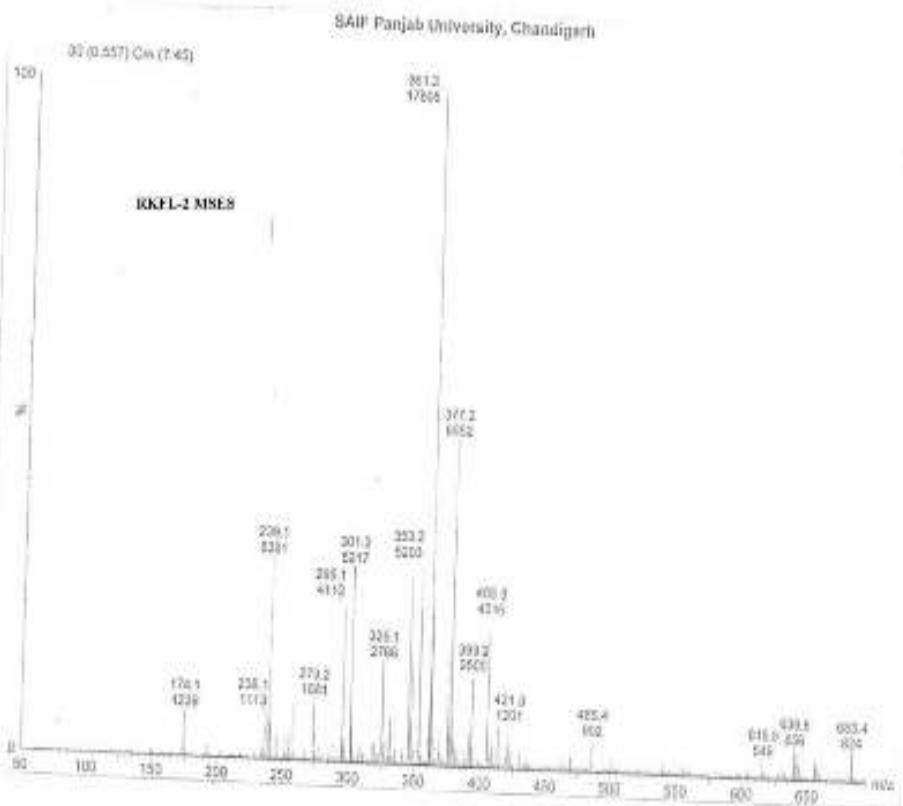
RKFL-1 ^1H NMR

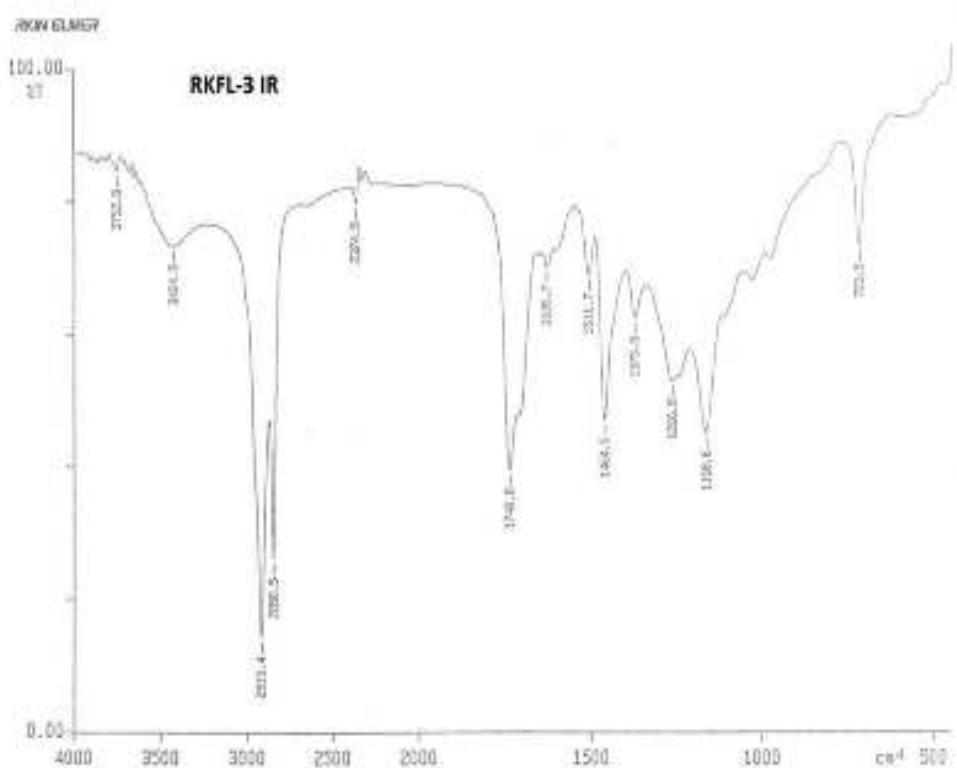
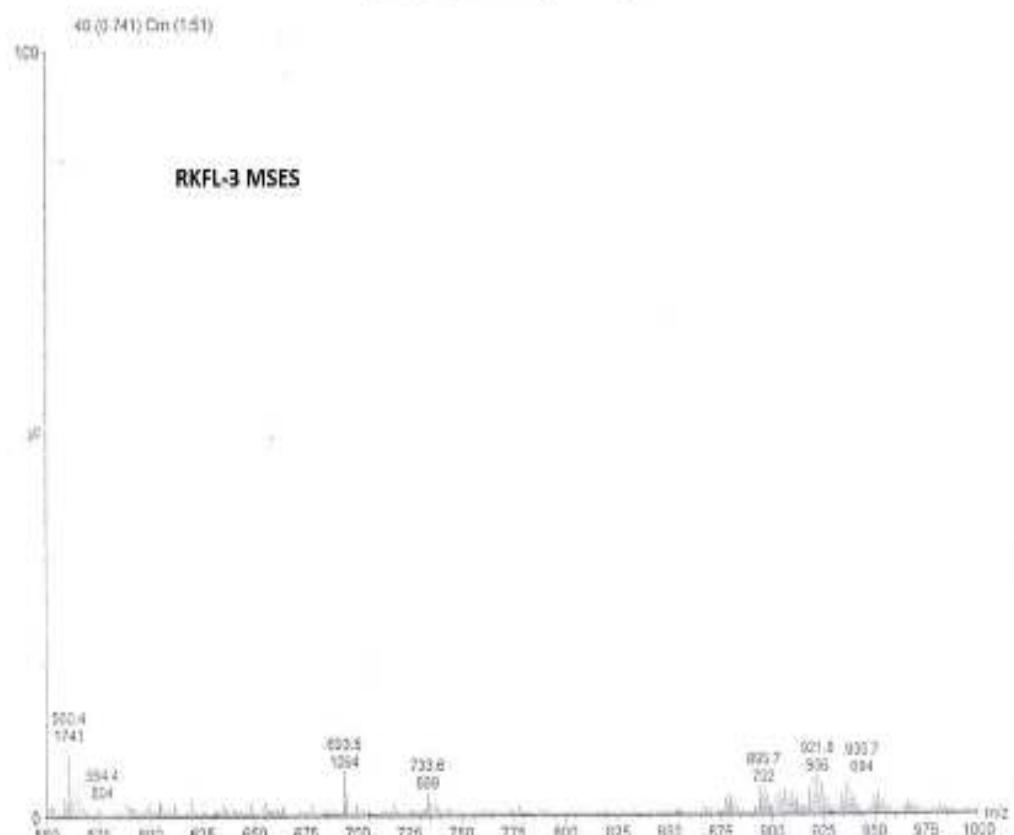


RKFL-1 ^{13}C NMR

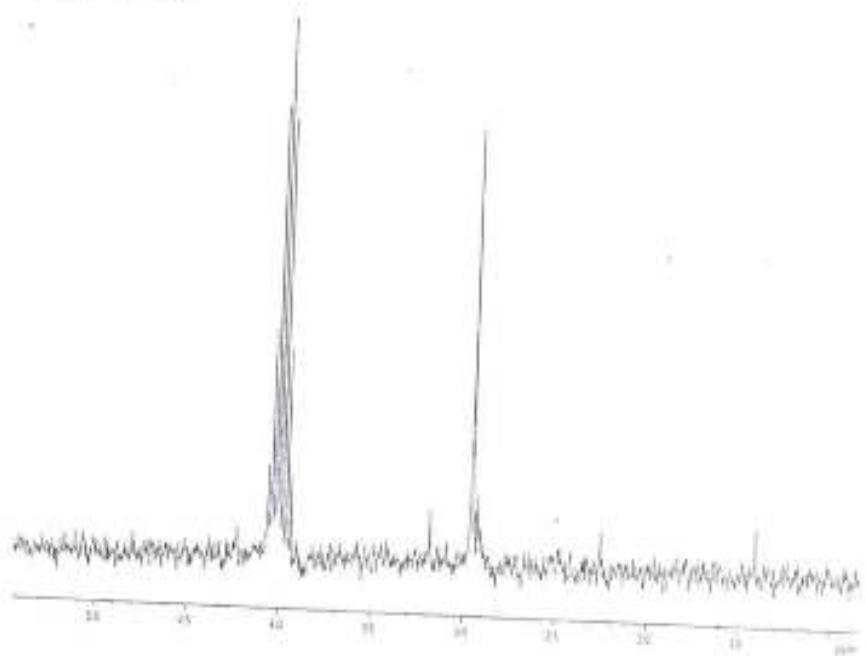








RKFL-3 ^{13}C NMR



RKFL-3 ^1H NMR

