PRELIMINARY PHYTOCHEMICAL STUDY AND ANTI-ARTHritic POTENTIAL OF CURCUMA ZEDOARIA ROsc ROOT

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By

Mr. MADAN LAL KAUSHIK, M.Pharm.,
(Registration No. DOUN09012)

Under the Guidance of

Prof. (Dr). SUNIL S. JALALPURE M.Pharm., Ph.D.,

KLE University’s College of Pharmacy,
J. N. Medical College Campus, Nehru Nagar,
Belgaum-590010, Karnataka, India

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1. INTRODUCTION

Rheumatoid arthritis (RA) is one of the major autoimmune diseases of global prevalence\(^1\), which is associated with systemic inflammatory disorders that are characterized by progressive joint pain, destruction and deformity of joints\(^2\). It has worldwide prevalence of about 1% of the adult population. It affects woman more than men with an annual incidence of 3 per 10,000 adults\(^3-4\). Recently, a self reported data by a physician-diagnosed arthritis indicated that a third of American adults had chronic joint symptoms. In the same line, it has been estimated that up to 2030, about 41 million adults aged 65 and older will have arthritis and chronic joint symptoms\(^5\). It is accompanied by significant morbidity and mortality, depending on the severity of the disease at the beginning. The disability risk can be as high as 33% and mortality 52% more which is frequently a result of infection or circulatory disease. It is also expected to have a significant effect on the quality of life\(^6\).

Currently, pharmacological management of RA includes administration of non-steroidal anti-inflammatory drug (NSAID), steroids and disease modifying antirheumatic drugs (DMARD). The value of these drugs in treating RA is also limited due to their major side effects and their propensity to cause stomach ulcers, gastric bleeding and perforations, and it has also been warned that their long term use may face the risk of stroke and heart attack\(^5,7\).

However, many of the plants validated experimentally were used in arthritis, but showed no cure. *Curcuma zedoaria* Rosc is commonly known as white turmeric, consists of dried pieces of rhizome. It is a large perennial herb with underground tuberous root-stock, growing widely in eastern Himalayas and in moist deciduous forests of the central region of Karnataka and Kerala, also cultivated throughout India. Traditionally, the plant has been used in crippling arthritis and frozen joints\(^8\).
In the present study, Freund’s complete adjuvant induced arthritis in female Wistar rats as a model of rheumatoid arthritis was used due to its relevance for the study of pathological pharmacology and on the basis of recommendation by Food Drug Administration USA. The advocated potential of petroleum ether, chloroform, methanol, ethanol, aqueous extracts and its formulation of Curcuma zedoaria roots in arthritic rats was not tested rigorously by scientifically controlled experiments. Hence this study has been extensively used to analyze the acute anti-inflammation and long lasting anti-arthritic effects of root extracts of Curcuma zedoaria in adjuvant-induced arthritis in female Wistar rats. The separation, purification and characterization of potent extract by TLC, column chromatography, preparative TLC, high performance thin layer chromatography, high performance liquid chromatography, UV, IR, ¹HNMR, and LCMS were performed which have been responsible for these activities.

2. RESEARCH OBJECTIVES

- To prepare various extracts (petroleum ether, chloroform, methanolic, ethanolic and aqueous extracts) by soxhlet extraction and cold maceration technique
- Preliminary phytochemical investigations of various extracts of C. zedoaria root
- Anti-arthritic activity by Freund’s Complete Adjuvant (FCA) induced method
- Acute anti-inflammatory activity by Carrageenan and Histamine induced method
- Separation of active phytoconstituents from potent extracts with chromatographic techniques
- Characterization of separated phytoconstituents by spectroscopy
- Development of single herb formulations from C. zedoaria potent extract
- Evaluation of acute anti-inflammatory and anti-arthritic activity of single herb formulations
- Accelerated stability studies of herbal formulations
3. LITERATURE REVIEW

3.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of undetermined etiology involving primarily the synovial membranes and articular structures of multiple joints. The disease is often progressive and results in pain, stiffness, and swelling of joints. In late stages deformity and ankylosis developed\textsuperscript{10}. Rheumatoid arthritis is the most common systemic inflammatory disease and characterized by symmetrical joint involvement potentially resulting in progressive destruction of articular and periarticular structures with or without generalized manifestation\textsuperscript{11}. RA is estimated to have a prevalence of 1 to 2\% and does not have any racial predilections. It can occur at any age, with increase prevalence up to seven decades of life. The cause of RA is unknown. Epidemiology data suggests that\textsuperscript{1}, there is strong genetic linkage to the class II of major histocompatibility complex (MHC) region on chromosome 6 and associated with the non-MHC gene PTPN22, a phosphate that regulates T-cell activation. The sole environmental factor consistently associated with RA is cigarette smoking. In some patients there are circulating autoantibodies, such as rheumatoid factor (against the Fc region of the other antibodies) and anti-CCP (against citrullinated epitopes on post translationally modified proteins), whose presence is associated with a worse prognosis\textsuperscript{12}.

The presence of activated immune cells increased local level of cytokines and other inflammatory mediator (eg. IL-1, TNF-\textalpha) propagating this process and supporting pannus formation, proliferation and neovascularization, cartilage, bone erosion and eventually joint destruction. Many people with RA have a certain genetic marker called HLA-DR\textsubscript{4}. However, DR1 is more important in Indians\textsuperscript{13}. 
Management of RA—and current treatment option:

1) Disease-modifying antirheumatic drugs (DMARDs) (2) Nonsteroidal antiinflammatory drugs (NSAIDs) (3) Use of glucocorticoid therapy for rheumatoid arthritis (4) Biological therapies (5) Surgery. (6) Non Pharmacological approach.

Adverse effect of drugs used in the treatment of rheumatoid arthritis:

NSAIDs have common GI and renal toxicity dyspepsia, gastric erosions, and peptic-ulceration,

DMARDs : Macular damage, alopecia, infrequent, myelosuppression, hepatotoxicity,rare

But serious (even life-threatening) pulmonary toxicity, dysgeusia, proteinuria, myelosuppression

Glucocorticoid: Paraesthesia, tremor, headaches, hypertrichosis, gingival hypertrophy, nausea, hypertension, renal disease sepsis, hypertension, hyperglycemia.

Biological therapies also increase the risk of many cardiac complications, thromboembolic infections and malignancy.

3.2 Curcuma zedoaria Rosc Plant:

*Curcuma zedoaria* Rosc consist of dried pieces of rhizome a large perennial herb with underground tuberous rootstock, growing widely in eastern Himalayas and in the moist deciduous forests of the central region of Karnataka and Kerala.

3.2.1 Traditional uses

Diuretic, anti-allergic, anti-asthmatic, ulcer, menstrual disorders dropsy, anti-arthritic and anti-inflammatory.

3.2.2 Chemical constituents

Phytochemical constituents of rootstock contain an essential oil, a bitter resin, organic acid, gum, starch sugar, dihydrocurcumin, tetrahydromothxycurcumin, tetrahydro-bismethoxycurcumin, demothxycurcumin and bisdemothxycurcumin, furanodiene,
curcumenol, isocurcumenol, curcumenone and curcumin. “curzenone and dehydrocurdione” are responsible for anti-inflammatory activity.

3.2.3 Reported pharmacological activities

Scientific studies of this plant have been proved on antimicrobial, antifungal, antiamoebic, Larvicidal, analgesic, antinociceptive, antiallergic, antiulcer, hepatoprotective, antivenom, hemagglutinating, antimutagenic, anticancer and antioxidant activity.

4. MATERIALS AND METHODS

4.1 Pharmacognostic investigations

 ê Collection of selected plant and authentication: Roots of C. zedoaria Rosc were collected from Cochin, Kerala and authenticated from National Botanical Research India, Lucknow, India.

4.1.1 Standardization parameters

 ê Morphological / Organoleptic evaluation
 ê Determination of alcohol-soluble Extractive
 ê Determination of water-soluble Extractive
 ê Determination of Petroleum-ether soluble Extractive
 ê Loss on Drying (LOD)
 ê Determination of Acid-insoluble Ash
 ê Determination of Water-soluble Ash

4.2 Extraction

4.2.1 Preparation of petroleum ether, chloroform, methanol, ethanol aqueous extracts

The powdered material was subjected to successive extractions in an increasing order of polarity using petroleum ether (40°C-60°C), chloroform, methanol and ethanol in a soxhlet apparatus. Aqueous extract was obtained by cold maceration.
4.3 Preliminary Phytochemical Investigation of Extracts\textsuperscript{15}

Qualitative chemical test of petroleum ether, chloroform, methanol, ethanol and aqueous extracts of \textit{C. zedoaria} roots were subjected to detect the presence of various phytoconstituents by using following tests:

- Tests for carbohydrates, proteins, steroid, amino acids, tests for glycosides, saponin glycosides, flavanoids, alkaloids, tannins and phenolic compounds and terpenoids.

4.4 Animal Selection:

Female Wistar rats between 2 and 3 months of age weighing 160 ± 40 g were used which were obtained from the central animal house of Jawaharlal Nehru Medical College, Belgaum India. All animals were housed in an animal room under normal condition of 24±1\degree C, 12-h light and dark cycle and 55±5% humidity. The study designs were approved by the Institutional Animal Ethical Committee of K.L.E.’S College of Pharmacy, Belgaum, India. (Resolution No. 31/7/2010-13).

4.5 Acute Toxicity Studies\textsuperscript{17}

The acute oral toxicity studies were carried out according to the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guideline 425, received from CPCSEA.

4.6 Preparation of Drug and Animal Groups for Anti-arthritic and Anti-inflammatory Activities:

The animals were divided into 14 groups of six animals each. Mineral oil injected in left ankle joint of rats kept as normal group received normal saline and FCA injected group as control group also received normal saline, \((10 \text{ mg/kg. i.p.})\) Indomethacin was used as standard drug, \((200 \text{ mg/kg. p.o.})\) Rumalaya forte was used as a herbal standard drug (Marketed preparation for arthritic disease), \((200 \text{ and } 400 \text{ mg/kg. p.o})\) petroleum ether,
chloroform, methanol, ethanol and aqueous extracts of *C. zedoaria* were used as test drugs. Dose calculation was based on w/w of each extract. Each extract was dissolved in normal saline and triturated with 2% tween 60 to form a suspension and the dose calculation was based on w/w for each extract. 0.1 ml FCA and mineral oil were injected through intra-articular injection in left ankle joint of rats at 0 day. The animal groups are as follows.

**Normal Group**

Group-I: Treated with 5 ml/kg.p.o normal saline + 0.1 ml mineral oil

**Control Group**

Group-II: Treated with 5 ml/kg.p.o normal saline + FCA

**Standard groups:**

Group-III: Treated with (10 mg/kg.i.p. Indomethacin )+ FCA considered as [standard-I]

Group-IV: Treated with 200 mg/kg.p.o Rumalaya forte + FCA considered as [standard-II]

**Test groups:**

Group-V: Treated with (200 mg/kg.p.o petroleum ether extract) +FCA considered as [Pet. ether-I]

Group-VI: Treated with (400 mg/kg petroleum ether extract) +FCA considered as [Pet. Ether- II]

Group-VII: Treated with (200 mg/kg.p.o chloroform extract) + FCA considered as [CHCl₃-I]

Group-VIII: Treated with (400 mg/kg.p.o chloroform extract) + FCA considered as [CHCl₃-II]

Group-IX: Treated with (200 mg/kg.p.o. methanol extract) + FCA considered as [MetOH-I]

Group-X: Treated with (400 mg/kg.p.o. methanol extract) + FCA considered as [MetOH-II].

Group-XI: Treated with (200 mg/kg.p.o ethanol extract) + FCA considered as [EtOH-I]
Faculty of Pharmacy, KLE University

4.7 Evaluation of Anti-arthritic Activity

4.7.1 Induction of arthritis

Pre-induction baseline was taken prior to the injection of Freund’s Complete Adjuvant (FCA) measured by left paw volume of each animal at 0 day for the induction of arthritis in female Wistar rats. All the rats were anesthetized with thiopentone sodium 40 mg/kg.i.p. 0.1 ml mineral oil was injected into the left ankle joint of normal group of animals. 0.1 ml FCA was injected into the left ankle joint of control and drug-treated groups.

4.7.2 Measurement parameters

- **Rat paw edema** on 0 days and thereafter 3, 7, 14, 21, 28, 35, and 42 days of FCA post-inoculation.

- **Hematological profile**: Body weight was observed at 0, 3, 7, 14, 21, 28, 35, and 42 days. Physiological examinations such as Hemoglobin, Red blood cell, White blood cell and Erythrocyte Sedimentation Rate were observed at 42 days.

- **Biochemistry profile**: Aspartate amino transferase, Alkaline amino transferase, Blood urea nitrogen, Uric Acid, Creatinine, Total protein and Nitric oxide synthesis.

- **Assessment of vascular permeability**

- **Behavioral observations**

  **Open-field test**: All the animals were subjected to open-field test before the induction of arthritis and thereafter 3, 14, 21, 28, 35, and 42 days of post-inoculation of FCA.

Radiography examination

Histopathology examination

Organ to body weight ratio\textsuperscript{22}

4.8 Evaluation of Anti-inflammatory Activity of C. zedoaria Extracts

4.8.1 Carrageenan induced paw edema in rat\textsuperscript{23}

Before the experiment, food was withdrawn overnight but adequate water was given to the rats. Dose selected were 200 and 400 mg/kg for each extracts. The animals were divided into fourteen groups of 6 animals each. All the doses were given orally half an hour before the administration of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark in the plethysmometer.\textsuperscript{13} The paw volume was measured after (1 h) injection carrageenan and then every hour till 6 h of each group. The difference between the initial and subsequent reading gave the actual edema volume. The average paw swelling was calculated by comparing to normal group with control, standards and all treated groups compared with the control and percent inhibition of inflammation were calculated by using the standard formula,

4.8.2 Histamine–induced paw edema in rat\textsuperscript{23}

For the study of Histamine–induced paw edema, the animals were treated exactly with the same method as carrageenan induced model but instead of carrageenan, here 0.1 ml of 1% w/w histamine in normal saline was used. All carrageenan-induced inflammation methodology was adopted for histamine-induced inflammation.
4.9 Scheme: 1. for Separation of Curcuminoid from Petroleum Ether Extract of

*Curcuma zedoaria*²⁴

Separation of curcumin from Curcuminoid

Root of *C. zedoaria* → Soxhlet

Petroleum ether extract

TLC with solvent system chloroform, acetone, and ethanol (45: 45: 10)

Separated Curcuminoid

Separated by column chromatography with solvent system ratio of chloroform and methanol

Column

Fractions

Total

Fractions

150-1

12

83-105

106-114

115-150

13-22

23-82

83-105

Recrystallized and Dried

Comparison with standard curcuminoid on preparative thin layer chromatography with solvent system Chloroform acetone, and ethanol (45: 45: 10)

Recrystallized and Dried

HPLC  HPTLC

Spectroscopy

¹HNMR  LCMS  IR  UV
4.10. Development of Single Herb Formulation\textsuperscript{25-26}

Scheme: 2. Methodology for development of single herb formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Curcuma zedoaria root extracts</th>
<th>Ingredients</th>
<th>Quantity required of additives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light kaolin</td>
<td>Compound tragacant</td>
</tr>
<tr>
<td>Formulation A</td>
<td>Pet ether + CHCl\textsubscript{3} + EtOH extracts</td>
<td>0.6 g.</td>
<td>25%</td>
</tr>
<tr>
<td>Formulation B</td>
<td>Pet ether + CHCl\textsubscript{3} extracts</td>
<td>0.4 g.</td>
<td>25%</td>
</tr>
<tr>
<td>Formulation C</td>
<td>Pet ether + EtOH extracts</td>
<td>0.4 gm</td>
<td>25%</td>
</tr>
<tr>
<td>Formulation D</td>
<td>CHCl\textsubscript{3} + EtOH extracts</td>
<td>0.4 gm</td>
<td>25%</td>
</tr>
</tbody>
</table>

Procedure\textsuperscript{186}

The bioactive extracts are mixed with light kaolin in a mortar and compound powder of tragacanth is added. The orange syrup is added and triturated so as to form a smooth cream. The foreign particles are removed with the tip of the glass rod than the benzoic acid solution is incorporated and amaranth solution previously diluted with chloroform water is
added and stirred thoroughly so as to form a uniform mix. More orange syrup up to the required volume is added.

4.10 Acute Toxicity Studies of Herb Formulations

The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guideline 425.

4.11 Evaluation of Anti-arthritis and Anti-inflammatory Activities of Single Herb Formulations of *C. zedoaria* Root Extracts

The rats were divided into eight groups of six animals in each group. Normal, control and standard-I and standard-II groups were selected from our previous studies. Potential effect of *C. zedoaria* root extracts on arthritic and acute inflammation in rats were studied.

4.11.1 Preparation of test sample and animals groups for anti-arthritis and acute anti-inflammatory activities.

**Normal Group:** Treated with (5 ml/kg.p.o) + normal saline + mineral oil

**Control Group:** Treated with (5 ml/kg.p.o) normal saline + FCA

**Standard group-I:** Treated with (10 mg/kg.i.p Indomethacin) + FCA considered as [standard-I]

**Standard group-II:** Treated with (200 mg/kg.p.o Rumalaya forte) + FCA considered as [standard-II]

**Formulation test groups**

**Group-I:** Treated with (200 mg/kg, p.o) single herb Formulation-A +FCA considered as [SHF-A]

**Group-II:** Treated with (200 mg/kg, p.o) single herb Formulation-B+ FCA+ considered as [SHF-B]
Synopsis

Group-III: Treated with (200 mg/kg, p.o) single herb Formulation-C+ FCA+ considered as [SHF-C]

Group- IV: Treated with (200 mg/kg,p.o) single herb Formulation-D+FCA considered as [SHF-D]

4.11.2 Evaluation of anti-arthritic and anti-inflammatory activities of formulations were followed using the previous methodology

4.12 Accelerated Stability Studies of Single Herb Formulations of C. zedoaria

The accelerated stability studies were carried out for single herb formulations of bio-active constituents at Temperature 40 °C± 2 °C at 80 % humidity. The stability was studied for the period of three months. The different parameters such as colour, odour, viscosity, pH, and sedimentation volume and redispersibility test were studied for all the formulations at 1st, 2nd and at 3rd months.

5. OVER VIEW OF THE RESEARCH WORK UNDERTAKEN

5.1 Successive Extraction

Successive extractions were performed with petroleum ether, chloroform, methanol, and ethanol. Aqueous extracts were obtained by cold maceration. The percentage yield and colour were obtained after successive extraction with petroleum ether (40-60° C): 7.26 % w/w dark brown, chloroform: 2.60 w/w dark golden, methanol: 10 % w/w blackish, ethanol: 5.72% w/w reddish brown and aqueous: 20% w/w brownish yellow.

5.2 Preliminary Phytochemical Investigation

Preliminary phytochemical data revealed that petroleum ether, chloroform and ethanol extracts of C. zedoaria showed the presence of steroids, terpenoids, glycosides, alkaloids, tannins and other phenolic compounds (curcuminoid).

5.3 Acute Toxicity Study
Acute toxicity studies showed the non-toxic nature of each extract of *C. zedoaria*. There was no lethal or any toxic reactions observed at 2000 mg/kg. Hence, 1/10th of the lethal dose were taken as effective dose (therapeutic dose) LD₅₀ cut off value is 200 and 1/5th double of this 400 were selected for further study.

5.4 Anti-arthritic Activity

Intra-articular injection of FCA-induced arthritic model was suitable for long testing anti-arthritic activity of *C. zedoaria* root extracts. The petroleum ether, chloroform and ethanol extracts at dose 200 and 400 mg/kg showed anti-arthritic activity.

In the present study, the FCA-induced chronic inflammation in joints of control group is manifested as a progressive increase in paw edema. It is noteworthy that the inhibitory effect of petroleum ether, chloroform, and ethanol extracts of *C. zedoaria* roots in rat paw edema were observed from third day to the last day of study and recovery in health status such as body weight, ESR, Hb, RBC and WBC. Moreover, reduction was seen in the nitric oxide level and vascular permeability. In addition, protective effects were observed in rat joints. The result of behavior studies confirms the improvement in the anxiety, and abnormality in mobility. However, methanol and aqueous 200 and 400 mg/kg treated groups have failed in these aspects. On the basis of biochemical data it was observed that no toxic effect was found in any of the *C. zedoaria* extract. The organ to body weight ratio showed improvement in the change in organ weight in petroleum ether, chloroform and ethanol groups.

5.5 Acute Anti-inflammatory Activity

Petroleum ether, chloroform and ethanol extracts at both doses of *C. zedoaria* showed significant reduction in carrageenan and histamine induced paw edema in rats from 2nd hours to 6th hour of the study. However, methanol and aqueous extract showed no significant reduction in paw edema in rats.
Hence petroleum ether, chloroform and ethanol extracts showed potent acute anti-inflammatory and anti-arthritic effects.

Amongst the three extracts, petroleum ether extract showed the highest effect on acute anti-inflammatory activity. So, the further phytochemical study of petroleum ether extract of *C. zedoaria* was carried out.

### 5.6 Isolation and Characterization of Active Constituent from Potent Extract of *C. zedoaria* root

On the basis of preliminary phytochemical data, petroleum ether extract showed the presence of steroids, terpenoids, and phenolic compounds (curcuminoids: curcumin, bismethoxy-curtuminoid and dimethoxycurtuminoid). On the basis of literature survey, these active constituents are responsible for anti-arthritic and acute anti-inflammatory activities. Phytochemical study of steroids and terpenoids of petroleum ether extract of *C. zedoaria* was already carried out.

In present the study the crude curcuminoid was separated and identified from petroleum ether extract of *C. zedoaria* by the comparison with herbal standard curcuminoid using preparative thin layer chromatography technique. The separation of curcumin from crude curcuminoid which obtained from petroleum ether extract of *C. zedoaria* by using column chromatography were identified by TLC, HPLC and HPTLC techniques. Separated fractions were collected and recrystalised and then subjected to spectroscopy analysis UV, IR, LCMS, and $^1$HNMR. On the basis of spectroscopy data it indicates that the separated compound was curcumin.

### 5.7 Acute Toxicity Studies of Herbal Formulations

All formulations were subjected to acute toxicity study. Acute toxicity studies of formulations showed no sign of toxicity at the dose of 2000 mg/kg.p.o. Hence 1/10th of the
lethal dose was selected to each formulation for anti-arthritic and acute anti-inflammatory activity.

5.8 Ant-arthritic Activity of Herbal Formulations

The single herb formulation from potent extracts obtained from *C. zedoaria* root was developed. Pharmacological evaluation of anti-arthritic activity of *C. zedoaria* extracts showed that petroleum ether, chloroform, and ethanol root extracts have potent anti-arthritic and anti-inflammatory of *C. zedoaria*. These three extract were mixed with additives in a standard ratio and prepared in a suspension form and named as SHF-A, SHF-B, SHF-C and SHF-D.

In the present study, SHF-A, SHF-C and SHF-D at 200 mg/kg showed significant reduction in the FCA-induced paw edema in rats from third day to last day of study compared to control group paw edema and recovery was observed in health status such as body weight, ESR, Hb, RBC and WBC. Moreover, reduction in nitric oxide level and vascular permeability was observed. In addition, protective effects were observed in rat joint and behavior studies which confirm its ability to overcome stress, anxiety and abnormality in mobility. However, SHF-B treated groups showed non-significant results in these aspects. Biochemical and organ to body weight ratio studies indicate that no toxic effects were observed in any formulations but SHF-A showed highly potent anti-arthritic activity. Formulations SHF-A, SHF-C and SHF-D at 200 mg/kg showed anti-arthritic activity.

5.9 Anti-inflammatory Activity of Formulation

Formulations SHF-A, SHF-C and SHF-D at 200 mg/kg dose of *C. zedoaria* showed significant reduction in the paw edema from 2nd hour to 6th hour of the study. However, SHF-B showed less significant effect in the reduction of carrageenan and histamine induced paw edema in rats. SHF-A, SHF-C and SHF-D showed acute anti-inflammatory activity.

5.10 Accelerated Stability Study
All formulations were stable at both environment room temperature and 40 °C

6. CONCLUSION

It can be stated that the petroleum ether, chloroform and ethanol extracts of C. zedoaria root and its formulations SHF-A, SHF-C and SHF-D has beneficial effects in long lasting reduction in rat paw edema, recovery in hematological changes, inhibitory effects on nitric oxide synthesis and vascular permeability. It also showed the protective effect on arthritic rat joints without any toxic effect. The mechanism may be mediated via the inhibition of prostaglandin synthesis as well as central inhibitory mechanism, this, justifying the claim made by Siddha and Ayurvedic classics.

7. BIBLIOGRAPHY


## List of Publications related to Ph.D. research work

<table>
<thead>
<tr>
<th>S. no</th>
<th>Title of research articles</th>
<th>Name of Journal</th>
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<tr>
<td>1</td>
<td>Anti-inflammatory effect of ethanolic and aqueous extracts of <em>Curcuma zedoaria</em> Rosc root</td>
<td>International Journal Of Drug Development And Research</td>
<td>Elsevier, embase, scopus, Chemical abstract services, Open-J-Gate, DOAJ, CAB Abstract</td>
<td>Kaushik M and Jalalpure S</td>
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<td>3</td>
<td>Effect of root extracts of <em>Curcuma zedoaria</em> Rosc on behavioral and radiology changes in arthritic rats</td>
<td>Advance Journal in Pharmaceutical and Technology Research</td>
<td>CAB Abstract, caspur, chemical abstract, DOAJ, EBSCO, Scopus, SIIC database</td>
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<td>4</td>
<td>Comparative efficacy of ethanolic and aqueous extracts of <em>Curcuma zedoaria</em> Rosc root on behavioral and radiology changes in arthritic rats</td>
<td>International Journal of Pharmaceutical Research</td>
<td>Chemical abstract, services embase, scopus, International periodic dictionary and Indian chemical society.</td>
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