CHAPTER 3: REVIEW OF LITERATURE

3.1 Historical Perspective of Rheumatoid Arthritis (RA)

The first known traces of arthritis date back at least as far as 4500 BC have shown some signs of RA. So far, that has been the earliest appearance of this condition. The first documented appearance of RA came much later in 123 AD in India. A text called the Charaka Samhita contains a description of what sounds like RA, painful joints in both hands and feet, eventually spreading to other areas of the body. There was also recorded of occasional fever and appetite loss. In 1591 A French physician, Guillaume de Baillou, wrote one of the first books on the arthritis disease. He uses the term “rheumatism” to give description to an ailment with inflammation, stiffness in muscles, and pain in the joints. “The whole body hurts, in some the face is flushed; pain is most severe around the joints, so that the slightest movement of the foot, hand or fingers causes a cry of pain…At night…the pain becomes more serious and the patient cannot sleep. In 1680 A Peruvian bark containing quinine, an anti-malarial agent was being used to treat the symptoms of rheumatism. In 1763 another bark was discovered to produce positive results in fighting the effects of rheumatism; willow bark contains salicylate, the main ingredient for aspirin. The first recognized description of RA was in 1800 by the French physician Dr Augustin Jacob Landré-Beauvais (1772–1840) who was based in the famed Salpêtrière Hospital in Paris. In 1859 The name RA itself was coined by the British rheumatologist Dr. Alfred Baring Garrod. In 1859 the term RA wasn’t coined until a London physician, Sir Alfred Garrod, came up and that was the start of RA being recorded in medical books and records. In 1893 a joint replacement surgery had begun with a carbon steel screw and plate system developed by W.A.
In 1895 the X-ray was developed which allowed physicians to see actual damage and inflammation of an arthritis sufferer.

In 1897 aspirin was manufactured by the company Bayer, using the willow bark substance, discovered over a century before. It fast became known worldwide as a standard treatment for pain and RA. In 1927 gold salts began to be injected into patients periodically to relieve muscle pain, associated with RA. In 1929 the first artificial hip joint was patented. In 1939 down in Melbourne Australia, Sir McFarlane Burnet introduced the first autoimmunity theory. This is the theory that antibodies cause the body’s immune system to malfunction and attack its own tissues. In 1940 RA gained more recognition. In 1941 American Rheumatism Association was formed. In 1946 the American Committee to Control Rheumatism was founded and two years later the Arthritis Foundation began. In 1948 most important discoveries were uncovered by Dr. E.C. Kendall and Dr. Philip Hench to show the world that steroid hormones can be used as therapeutic anti-inflammatory. They win the Nobel Prize! 1948 a discovery just as important was made known; a test called the Rose-Waaler diagnostic test, was developed which revealed the antibody known as the rheumatoid factor found only in the blood of people with RA.

**Surgeries were discovered.** The next fifty years of the century saw many new treatments, procedures and discovered surgeries. In 1955 Prednisone, a synthetic off shoot of cortisone was ushered in and began the most popular oral corticosteroid medication. Plaquinil was also introduced as the new anti-malarial drug used to put the RA into remission.
3.1.2 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 develop.

3.1.3 Epidemiology

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. 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 increased prevalence up to seven decades of life. The disease is three times more common in woman, predominated by a ratio of 6:1; the sex ratio is approximately equal among patient in the first decade of life and in those more than 60 years old. The age of onset is typically around 30 to 60 years and reaches its peak in the fourth decade. Patient with RA have 6 times the probability of severe on the activity, 4 times as many as restricted days and 10 times the work disability in the general population. After 10 years of the disease duration, more than 50% of patients are unable to work at all. Survival rates among patient with RA are lower than those in the general population 40 to 60% of the patient with advanced RA survive 5 years or less, following diagnosis and die 10 to 15 years earlier than expected.
3.1.4 Socio-Economic Impact of RA

RA is a disease that is associated with major socio-economic implications for the population it affects. The articular and extra-articular progressive nature of RA leads to both significant patient morbidity and mortality\textsuperscript{37-38}.

3.1.5 Etiology

The cause is unknown. On epidemiology data suggest that\textsuperscript{35}, 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{12,39-40}.

The presence of activated immune cells increased local level of cytokines and other inflammatory mediator eg. IL-1, TNF-α propagating this process and supporting pannus formation, proliferation and neovascularization, cartilage, bone erosion and eventually joint destruction\textsuperscript{38,41}.

Evidence for the importance of genetic susceptibility comes from higher concordance rate in monozygotic than in dizygotic twins (3%) and increased frequency of disease in first degree relative of patients with RA. Many people with RA have a certain genetic marker called HLA-DR\textsubscript{4}. However, DR\textsubscript{1} is more important in Indians\textsuperscript{38-42}.

More specifically disease susceptibility is associated with a shared epitope (SE) of the specific amino acid sequence of the β 1 chain of a number of class II
alleles located in the third allelic hypervariable region of HLA \( \beta_1 \), between amino acid residues 67 and 74 which flank the T cell recognition site. Female gender is risk factor and this susceptibility is increased post-partum and by breast feeding\(^{38}\).

### 3.1.6 Pathology

The final common pathway of joint destruction involves pro-inflammatory cytokines and tissue-destructive enzymes (matrix metalloproteinases) produced T cells, macrophages, type B synoviocytes and bone destructive osteoclasts. Pro-inflammatory cytokines include tumor necrosis factor -interleukin IL–16, IL–15 and IL–17\(^{12-42}\).

A number of cell types have been proposed to orchestrate the pathology of RA. T cells provide stimulation of B cells and macrophages. In addition to soluble mediators, cell – cell contact is also important: T cell macrophage contact is important for Tiff secretion. Conventionally, T cells are activated by dendritic cells although it is now appreciated that B cells also secrete cytokines and chemokines, and are effective antigen presenting cells that can maintain T–cell activation in the synovium\(^{42}\).

Rheumatoid synovium is heavily infiltrated with lymphocytes. Most of these are activated CD4 helper T cells. Activated CD4 cells are a well-known source of cytokines, which activate other immune cells as well as macrophages\(^{43}\). The latter in turn themselves secrete a variety of proinflammatory and tissue-degrading factors. The rheumatoid synovium is embarrassingly rich in both lymphocyte and monocyte-derived cytokines. The activity of these cytokines can account for many features of rheumatoid synovitis. Not only the cytokines pro-inflammatory, but some, such as IL-1 and TGF cause proliferation of synovial cells and fibroblasts. They also
stimulate synovial cells and chondrocytes to secrete proteolytic and matrix degrading enzymes\textsuperscript{42-44}.

Although T cells play a primary role in the pathogenesis of RA and B cells are also involved. Approximately 80\% of patients have rheumatoid factors (RF) which are auto antibodies directed against the Fc portion of IgG present in serum and synovial fluid. The significance of circulating RFs in the pathogenesis of RA is uncertain, but their presence in the joints is believed to contribute to the inflammatory reaction. Synovial fluid IgG rheumatoid factors self-associate (IgG-anti-IgG) to form immune complexes that fix complement, attract polymorph nuclear leukocytes, and lead to tissue injury by a type III hypersensitivity reaction\textsuperscript{42,45}.

3.1.6.1 Current Ideas on The Pathogenesis of Rheumatoid Arthritis

During the development of RA synoviocytes (particularly type B) become hyperplastic, and there is an influx of inflammatory cells. A number of micro-environments develop.

(a) Conventional antigen presentation of putative auto antigens occurs in the regional lymph nodes, but myeloid type antigen presenting cells are also present in the inflamed synovium. Co-stimulation is provided by B 7.1 and B 7.2 on the APC interaction with CD28 on the T cell, and by CD40 ligand (CD40). On the APC interacting with T-cell CD40. (CTLA4-lg (abatacept) inhibits the former interaction\textsuperscript{46}. The type of immune response generated also depends on the cytokine microenvironment. Secretion of IL-12 by APC favours a T\textsubscript{H}1 immune response with production of interferon–γ (INFγ) and tumour necrosis factor-α (TNF-α). Recently, the combination of IL–6 and transforming growth factor-β (TGF-β), has been implicated in the differentiation of pro-inflammatory T\textsubscript{H}17 cells, that contribute to autoimmune disease.
(b) Macrophages interacting with activated T cells secrete pro-inflammatory cytokines such as TNF-α and IL-1β. Membrane-membrane interactions are important in this cellular crosstalk, which is enhanced by T cell stimulation with macrophage derived IL-15.

(c) B cells are also present in large number and in distinct patterns. Follicular synovitis germinal centers develop similar to those represented in lymph nodes, with B and T cells surrounding follicular dendritic cells (FDC). In aggregate synovitis, FDC is absent but T and B cells remain clustered. In diffuse synovitis, the cells are more loosely mixed.
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KLE University  15

Figure: 2 Basic pathology of Rheumatoid arthritis

Figure: 3 Current ideas from the pathogenesis of rheumatoid arthritis
The significance of these patterns is unclear but, supported by growth factor such as B lymphocyte stimulator and a proliferation-inducing ligand, B cell can perform a number of roles: autoantibodies production; as APC for T cell; and chemokine and pro-inflammatory cytokine secretion.

(d) One model of RA suggests that type B synoviocytes are the primary pathogenic cells and that immune mechanism have a lesser role. Even in immune models, however, synoviocytes seem to have an active role. In part stimulated by lymphotoxin-β (LT-β, produced by B cells and other cells), they regulate surface adhesion molecules and secrete pro-inflammatory mediators, matrix degrading enzymes and chemokines. In this way, they attract inflammatory cells into the joint and provide the niches to support the above microenvironments, while synergizing with inflammatory and destructive mechanisms $^{12,47}$.

### 3.1.7 Clinical Features

Symptoms include inflammation of the joints, swelling, pain and difficulty in moving which also involves of small joints of hands, wrists and the feet. The elbows, shoulders, hips, knees, and ankles may also be affected. Other symptoms include, loss of appetite, fever, loss of energy (weakness), anemia, myalgia, and joint stiffness which is typically worse in the morning and usually lasts atleast 1 hour before maximal improvement is seen for the day. Chronic joint deformities commonly involve subluxations of the wrists, metacarpophalangeal (MCP), and proximal inter phalangeal (PIP) joints. Extra articular involvement may include subcutaneous nodules, vasculitis, pleural effusion, pulmonary fibrosis, ocular manifestations, pericarditis, cardiac conduction abnormalities and bone marrow suppression. Stiffness and myalgias may precede development of synovitis $^{38,48}$. 
3.1.8 Classification criteria of Rheumatoid arthritis:49,51

1. Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.

2. Arthritis of 3 or more at least 3 joint areas have simultaneously soft joint areas tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.

3. Arthritis of hand joints at least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint.

4. Symmetric arthritis simultaneous involvement in the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry).

5. Rheumatoid nodules subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician.

6. Serum rheumatoid factor demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.

7. Radiographic changes typical of RA on posteroanterior hand and wrist radiographs, which must include erosion unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify) PIP: Proximal inter-phalangeal; MCP: Metocarpophalangeal.
3.1.9 Baseline Evaluation of Patients with Rheumatoid Arthritis

1) Subjective

Degree of joint pain
Duration of morning stiffness
Presence or absence of fatigue
Limitation of function

2) Physical examination

Documentation of actively inflamed joints
Documentation of mechanical joint problems: loss of motion, crepitus, instability, malalignment and/or deformity
Documentation of extra articular manifestations

3) Laboratory

Erythrocyte sedimentation rate/C-reactive protein
Rheumatoid factor
Complete blood cell count
Electrolytes
Creatinine
Hepatic panel
Urinalysis
Synovial fluid analysis
Stool guaiac

3.1.10 Radiography

Radiography of selected involved joints, section performed only at baseline to establish the diagnosis; may be repeated 6-12 months after disease onset if negative initially. Performed at baseline to assess organ dysfunction due to co-morbid
diseases, before starting medications. Performed at baseline if necessary, to rule out other diseases; may be repeated during disease flares to rule out septic arthritis 52-53.

3.1.11 Management of RA—And Current Treatment Option

There is no known cure for RA or means of preventing it. Optimal management requires early diagnosis and timely introduction of agents that reduce the probability of irreversible joint damage. While the ultimate goal of treating RA is to induce a complete remission, this occurs only in rare cases. Complete remission is defined as the absence of 1) symptoms of active inflammatory joint pain (in contrast to mechanical joint pain), 2) morning stiffness, 3) fatigue, 4) synovitis on joint examination, 5) progression of radiographic damage on sequential radiographs, and 6) elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) level -13. If complete remission is not achieved, the management goals are to control disease activity, alleviate pain, maintain function for essential activities of daily living and work, maximize quality of life, and slow the rate of joint damage. The initial evaluation of the patient with RA should document symptoms of active disease. Careful history taking, a complete review of systems, and a thorough physical examination (both general and musculoskeletal) are necessary. The severity and duration of morning stiffness and constitutional symptoms such as fatigue should be recorded. Laboratory evaluations should include complete blood cell count (CBC), platelet count, chemistry profile, RF measurement, and measurement of ESR or CRP 54.
1) Nonsteroidal antiinflammatory drugs (NSAIDs)

The initial drug treatment of RA usually involves the use of NSAIDs to reduce joint pain and swelling and improve function. There are no significant differences in efficacy among the NSAIDs, although there are some differences in the incidence of side effects. The frequency of some side effects may vary among different NSAIDs. Combinations of 2 or more NSAIDs should be avoided since they are no more effective and may have additive adverse effects\textsuperscript{53-54}. 

\textit{Guidelines for the Management of Rheumatoid Arthritis
2) Disease-modifying antirheumatic drugs (DMARDs)

All patients whose RA remains active despite adequate treatment with NSAIDs are candidates for DMARD therapy. Active RA may lead to irreversible joint damage even in the early months of the disease while NSAIDs and glucocorticoids may alleviate symptoms while joint damage may occur and progress. DMARDs have the potential to reduce or prevent joint damage, preserve joint integrity and function, and, ultimately, to reduce the total costs of health care and maintain the economic productivity of the patient with RA. These drugs include hydroxychloroquine (HCQ), sulfasalazine (SSZ), methotrexate (MTX), gold salts, D-penicillamine (DP), and azathioprine (AZA). DMARDs have common characteristics. All are relatively slow acting, with a delay of 1-6 months before a clinical response is evident. Efficacy cannot be predicted for the individual patient, but up to two-thirds of patients may have a response to these agents. Each DMARD has specific toxicity that requires careful monitoring.\textsuperscript{53-54}

3) Use of glucocorticoid therapy for rheumatoid arthritis

Treatment of the most symptomatic joints early in the disease course treatment of flares in 1 or a few joints, recovery of lost joint motion low-dose oral glucocorticoid (\(<=10 \text{ mg prednisone daily or equivalent}\)) and local injections of glucocorticoid are highly effective for relieving symptoms in patients with active RA\textsuperscript{53-54}.

4) Biological therapies

Biological therapies have brought new promise and opportunity to the practice of Rheumatology during the past decade. Number of treatment for RA have been developed Biological agents include:

- Tumor necrosis factor (TNF\(\alpha\)) blockers – etanercept (Enbrel), Infliximab (Remicade), Adalimumab (Humira)
Interleukin-1 blockers – Anakinra
anti-B cell (CD20) antibody – Rituximab (Rituxan)

Blockers of T cell activation - Abatacept (Orencia) \(^{54-55}\).

4) Surgery

Even if joint inflammation is successfully controlled or eliminated with medication, patients with chronic RA may be symptomatic from joint damage. If, in spite of optimal medical treatment, the patient has unacceptable pain and/or limitation of function because of structural joint damage, surgery should be considered. The most successful surgical procedures for RA are carpal tunnel release, resection of the metatarsal heads, and total hip and total knee arthroplasty. Outcomes of surgery and complication rates are related to the volume of surgery (i.e., number of surgeries performed annually at an institution), timing of surgery, the experience of the surgeon, patient's preoperative medical status, and postoperative management and rehabilitation. Occupational and physical therapy expertise is an important component of restoring and optimizing function, especially after total knee arthroplasty, shoulder surgery, and hand surgery \(^{53, 56-57}\).

5) Non pharmacological approach

Non pharmacological methods such as physiotherapy, occupational therapy and electrotherapy not only play a vital role in acute flares and in chronic stage but also helpful in relieving symptoms and protecting the joints from further damage. Patients with severe disease may benefit from surgical procedures such as tenosynovectomy, tendon repair and joint replacement \(^{38}\).
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3.1.12 Adverse Effect of Drugs Used in the Treatment of Rheumatoid Arthritis

1) **Non steroidal anti-inflammatory drug**

Salisylate--NSAIDs has common GI and renal toxicity dyspepsia, gastric erosions, peptic ulceration, small bowel inflammation and bleeding perforation haematemesis or melaena, occult GI blood loss and anemia.

2) **Disease-modifying antirheumatic drugs (DMARDs)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydroxychloroquine</strong></td>
<td>Macular damage</td>
</tr>
<tr>
<td><strong>Sulfasalazine</strong></td>
<td>Myelosuppression,</td>
</tr>
<tr>
<td><strong>Methotrexate</strong></td>
<td>Alopecia, infrequent, myelosuppression, hepatotoxicity, rare but serious (even life-threatening) pulmonary toxicity</td>
</tr>
<tr>
<td><strong>Injectable gold salts</strong></td>
<td>Myelosuppression, thrombocytopenia</td>
</tr>
<tr>
<td><strong>Oral gold</strong></td>
<td>Same as injectable gold but less frequent, plus frequent</td>
</tr>
<tr>
<td><strong>Azathioprine</strong></td>
<td>Myelosuppression, infrequent hepatotoxicity, early flu-like Illness elevated LFTs</td>
</tr>
<tr>
<td><strong>D-penicillamine</strong></td>
<td>Dysgeusia, proteinurea, myelosuppression,</td>
</tr>
<tr>
<td><strong>Auranofin</strong></td>
<td>Leucopenia</td>
</tr>
<tr>
<td><strong>Leflunomide</strong></td>
<td>Alopecia, Nausea, Leucopenia, Hepatitis, Thrombocytopenia</td>
</tr>
</tbody>
</table>

3) **Glucocorticoid therapy for rheumatoid arthritis**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclosporin</strong></td>
<td>Paraesthesia/tremor/ headaches, Hypertrichosis, Gingival hypertrophy, Nausea, Hypertension, Renal disease Sepsis</td>
</tr>
<tr>
<td><strong>Glucocorticoids</strong></td>
<td>Hypertension, Hyperglycemia&lt;sup&gt;53-54&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
4) **Adverse effects of biological therapy**

The increased incidence of untoward effects of biological therapies mainly TNF-α inhibitor has increased susceptibility to tuberculosis infection, causes multiple sclerosis and invokes the de novo demyelation. These biological therapies at the same time increase the risk of many cardiac complications, thromboembolic infections and malignancy. Combining biological therapies might heighten the risk of infections and malignant complication, by targeting parallel redundant pathway⁵⁵⁻⁵⁷.
3.2 Review of *Curcuma zedoaria* Rosc Plant

3.2.1 Synonyms

Figure: 4 *Curcuma zedoaria* Rosc
3.2.2 Morphological Characteristics: Curcuma zedoaria Rosc root-stock tuberous, ovoide, has many palmately attached sessile, cylindrical, accessory tubers, up to 2.5 in diameter as well as many smaller oblong to fusiform succulents root tubers at the tips of oblong, stouts, fleshy, fibrous roots. There is no distinct aerial stem, but the shoot, which may reach a height of 3 ft, has a pseudo- stem formed by the long and closely overlapping sheathing bases of four to six broad, lanceolate, finely acuminate smooth leaves tapering to the base and often colored purplish along the center. The inflorescence is a spathe from about 15 to 30 cm in length; it arises from the rhizome separated from the leafy shoot. It is closely enveloped by a few green leaves-sheaths in its basal half. Its distal half terminates as a tufted spike covered with several imbricating, oblong bracts of which each of the lower ones subtends 3 or 4 sessile yellow flowers, while the upper bracts are sterile and colored.

3.2.3 Geographical distribution
Curcuma zedoaria Rosc consist of dried pieces of rhizome (family Zingiberaceae), a large perennial herb with underground tuberous root-stock, growing widely in eastern Himalayas and in the moist deciduous forest of the central region of Karnataka and Kerala, India and also cultivated throughout the country\textsuperscript{14}.

3.2.4 Traditional uses

Ayurvedic reference to the plant roots suggests that, Vadana satapana, Varanropan and Sotharn are used, which means analgesic, anti-inflammatory and wound healing properties, diuretic, anti-allergic, anti-asthmatic, antiulcer, menstrual disorders dropsy, vomiting and improves blood circulation.

Siddha refers to the Curcuma zedoaria it is a single herb whose roots have been used in crippling arthritis and frozen joints\textsuperscript{18}.

3.2.5 Reported pharmacological activities

The studies of this plant proved scientifically

1) **Antimicrobial activity**: The antimicrobial activity of extracts of C. zedoaria tubers was tested against six bacterial and two fungal strains using the agar well diffusion and broth dilution methods. Petroleum ether, hexane, chloroform, acetone and ethanol extracts exhibited antibacterial as well as antifungal activity\textsuperscript{61-64}.

2) **Antifungal activity**: The extract of C. zedoaria was found to have pronounced inhibitory activity against a wide variety of human pathogenic fungi, including strains resistant to the common antifungals amphotericin B and ketoconazole, thus proving the claims made by people of Kenyah (Indonesian Borneo) for the use as an antifungal agent\textsuperscript{65}.

3) **Antiamoebic activity**: Alcoholic extract of rhizome of C. zedoaria was able to inhibit the growth of Entamoeba histolytica at a concentration of 1–10 mg/ml\textsuperscript{66}.
4) Larvicidal activity: Zedoary oil and its formulated preparation, zedoary oil impregnated sand granules, were tested for larvicidal efficacy against *Aedes aegypti* mosquitoes and compared with that of Abate (temephos)\(^6\). 

5) Analgesic activity: The activity was investigated using several models of pain in mice, including writhing, formalin and capsaicin. Curcumenol presented promising analgesic effects, being several times more potent than the reference drugs\(^6\). 

6) Antinociceptive activity: The extracts obtained from mother rhizome collected in autumn and winter at doses of 10 mg/kg intraperitoneally caused considerable antinociceptive activity, inhibiting 91.1 and 93.4% of the abdominal constrictions, respectively, whereas compounds curcumenol and dihydrocurdione caused inhibitions of 64 and 46 %, respectively. These results confirm that both compounds contribute towards antinociceptive and analgesic activity\(^6\). 

7) Antialлерgic activity: The effect of four curcuminoids (curcumin, dihydrocurcumin, tetrahydrodemethoxycurcumin and tetrahydrobisdemethoxycurcumin) and several related compounds isolated therefrom were studied for degranulation. Among them, curcumin showed the highest activity against beta-hexosaminidase release having a 50% inhibitory concentration (IC\(_{50}\)) of 5.3 mM, followed by bisdemethoxycurcumin (IC\(_{50}\) 11 mM)\(^7\). 

8) Antiulcer activity: *C. zedoaria* is the chief ingredient in several Unani preparations used to treat peptic ulcer the results were comparable with that of the standard drug omeprazole (30 mg/kg, i.p.), thereby providing justification that the root is effective in affording protection against hyperacidity and gastric ulcers\(^7\).
9) **Platelet activating activity:** The freeze-dried form of aqueous extract of *C. zedoaria* was studied for its inhibitory effect on platelet activating factor using a radioligand. It was found that *C. zedoaria* inhibited 50.60% platelet activating factor binding to rabbit platelets at a concentration of 200 mg/ml.

10) **Hepatoprotective activity:** Hepatoprotective sesquiterpenes were isolated from the aqueous acetone extract of the rhizome of *C. zedoaria*. Principal sesquiterpenes, furanodiene, germacrone, curdione (25), neocurdione (26), curcumeneol, isocurcumenol, aerugidiol, zedoarondiol, curcumeneone and curcumin were found to show potent protective effect on D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced acute liver injury in mice.

11) **Antivenom activity:** The aqueous extract of *C. zedoaria* showed clear inhibitory activity. It was found that the extract targeted neurotoxin and protein-degrading enzyme present in the venom, thus suggesting the use of aqueous extract as antivenom.

12) **Hemagglutinating activity:** Hemagglutinating activity has been shown in the extract of the *C. zedoaria*. Crude proteins obtained by Mg/NP-40 extraction from Curcuma species exhibited agglutination activity against rabbit erythrocytes.

13) **Antimutagenic activity:** *C. zedoaria* was found to possess moderate activity against benzo[a] pyrene.

14. **Anticancer activity:** The inhibitory effect of water extract of *C. zedoaria* on experimental pulmonary metastasis of B16 melanoma cells was investigated. The water extract of *C. zedoaria* may play an important role in the inhibition of cancer metastasis.

15. **Antioxidant activity:** At 20 mg/ml, the essential oil of *C. zedoaria* was moderate to good in antioxidant activity, good in reducing power and excellence in scavenging...
effect on 1,1-diphenyl-2-picrylhydrazyl radical but low in chelating effect on ferrousion\textsuperscript{80}.

### 3.2.6 Phytochemical review of Curcuma zedoaria Rosc

**1) C. zedoaria** is a rich source of essential oils, starch, curcumin, arabin, gums, etc\textsuperscript{81}.

More than 10 sesquiterpenes have been isolated from the rhizome of *C. zedoaria* and 15 such compounds have been structurally characterised namely furanodiene, furanodienone, zedorone, curzerenone, curzeone, germacrone, 13-hydroxy germacrone, dihydrocurdione, curcumenone and zedoaronediol\textsuperscript{82}.

**2) Phytochemical analysis** was carried out by using air-dried rhizomes (3 kg). The powder was extracted twice with dichloromethane at room temperature for five days, and then with ethyl acetate and methanol, respectively. The extracts were then concentrated under reduced pressure to give the respective fractions. A part of the dichloromethane fraction (50 g) was chromatographed using a silica gel column eluted with a mixture of hexane–ethyl acetate in increasing polarity. The fraction F1 (3.5 g), obtained from the above, was rechromatographed over a silica gel column and, when eluted with benzene–acetone (9 : 1), yielded about 500 mg of compound 1 and 150 mg of compound 2. Spectroscopic data (IR and NMR) confirmed identity of compound 1 as curcumenol and compound 2 as a mixture of phytosterols (especially sitosterol and stigmasterol 2 : 1)\textsuperscript{68,83}.

**3) The seasonal variation** of curcumenol (12) and dihydrocurdione, two active terpenoids from different parts. The results showed that both terpenoids are present in all the parts studied. However, *C. zedoaria* exhibited about three times more terpenoids in the mother rhizome in autumn than in other parts and seasons studied. A new audesmane-type sesquiterpene, zedoarofuran, and six new guaiane or secoguaiane-type sesquiterpenes, 4-epicurcumenol, neocurcumenol, gajutsulactones
A and B and zedoarolides A and B, were isolated from the aqueous acetone extract of zedoaria rhizome together with 36 known sesquiterpenes and two diarylheptanoids. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence\textsuperscript{84}.

4) Two guaiane derivatives were isolated from the rhizomes of \textit{C. zedoaria}. Their structures, zedoalactone A and zedoalactone B, were established by 1H and 13C NMR spectroscopic studies and by comparison with the closely related compounds \textsuperscript{69,85}. Zedoarol, 13-hydroxygermacrone and curzeone were isolated and structurally elucidated by using \textit{C. zedoaria}\textsuperscript{86}.

5) Three sesquiterpenoids, curcumenone, curcumanolide-A and curcumanolide-B, were isolated from the dried rhizome of \textit{C. zedoaria}\textsuperscript{87}

6) Ethyl paramethoxycinnamate was isolated from the methanolic extract of \textit{C. zedoaria} by chromatography on neutral alumina and silica gel\textsuperscript{88}.

7) In the course of searching for biologically active sesquiterpenoids from the \textit{Curcuma} genus, two sesquiterpenoids were isolated from the rhizome of \textit{C. zedoaria}. Their structures were identified as a-turmerone and b-turmerone\textsuperscript{89}. The structural elucidation of these compounds was carried out by comparison of their physical and spectral data with previously reported values\textsuperscript{90}.

8) Essential oils were isolated from the rhizomes a total of 36 compounds but were only able to structurally characterize epicurzerenone and curzerene\textsuperscript{91}.

9) The essential oil obtained by hydrodistillation of the rhizome of \textit{C. zedoaria} native to north-east India has been analysed by gas chromatography (\textit{GC}) and gas chromatography–mass spectrometry (\textit{GC-MS}). Thirty-seven constituents representing about 87.7\% of the total oil have been identified. Curzerenone (22.3\%)
was the major component, followed by 1,8-cineole (15.9%) and germacrone (9.0%)\textsuperscript{92}.

10) The chemical investigation of essential oils of rhizomes of \textit{C. zedoaria}, done by GC and GC-MS, revealed the presence of 1,8-cineole (18.5%), cymene (18.42%), \alpha-\text{phellandrene} (14.9%) (27) and \textit{b}eudesmol (10.6%)\textsuperscript{93}.

11) The essential oil produced by hydrodistillation of \textit{C. zedoaria} leaves was investigated by GC and GC-MS. Twenty-three compounds were identified, accounting for 75% of the oil. The oil of \textit{C. zedoaria} was made up mainly of mono and sesquiterpenoids, monoterpane hydrocarbons (2.3%), oxygenated monoterpenes (26%), sesquiterpane hydrocarbons (38%) and oxygenated sesquiterpenes (13.5%). The major constituents of the leaf oil were a-terpinyl acetate (8.4%), isoborneol (7%) and dehydrocurdione (9%)\textsuperscript{94}.

12) Chemical analysis of the volatile oil from \textit{C. zedoaria} using GC-MS technique revealed the presence of \textit{b}-tumerone (19.88%), 1,8-cineole (8.93%) and zingiberene (23) (7.84%) as major constituents\textsuperscript{95}.

13) The essential oil of the dried rhizome was isolated using simultaneous steam distillation and solvent extraction and its fractions were prepared by silica gel column chromatography. In total, 36 compounds were identified in the essential oil, including 17 terpenes, 13 alcohols and 6 ketones. Epicurzerenone and curzerene were found in the first and second highest amounts (24.1 and 10.4%)\textsuperscript{90}. Curcumin, dihydrocurcumin, (24 tetrahydrodemethoxycurcumin and tetratetrahydrobisde methoxycurcumin were isolated together with two bisabolane-type sesquiterpenes from 80% aqueous acetone extract of the rhizome of \textit{C. zedoaria}\textsuperscript{80}.

14) Bioassay-directed fractionation of an ethanol extract of \textit{C. zedoaria} led to the isolation of an active curcuminoid, which was identified as demethoxycurcumin by
comparison of its 1H and 13C NMR spectra with literature data and by direct comparison with synthetic material. Curcumin and bisdemethoxycurcumin were also obtained.

15) The variation of curcuminoid in the ethanolic extract of _C. zedoaria_ was measured by using high-performance liquid chromatography (HPLC). The analysis was carried out and Ethnomedicinal uses of _C. zedoaria_ for 425 nm using a BDS Hypersil C18 column as a stationary phase, 0.1% acetic acid aqueous solution and acetonitrile as mobile phase.

16) Ethanolic extracts of _C. zedoaria_ rhizomes collected from various parts of Thailand contained curcumin, demethoxycurcumin and bisdemethoxycurcumin in the range of 1.46 ± 0.45 to 5.73 ± 0.11% w/w (average 2.73 ± 1.24% w/w), 3.15 ± 0.15 to 10.98 ± 0.28% w/w (average 7.37 ± 2.71% w/w) and 0.49 ± 0.02 to 2.99 ± 0.20% w/w (average 1.40 ± 0.82% w/w), respectively. The highest average total curcuminoid content in the extracts was found to be 16.83 ± 0.62% w/w while the lowest content was 6.09 ± 1.79% w/w. This information will be useful as a guide for further standardization of _C. zedoaria_ extracts for which the content has not been reported elsewhere.

3.2.7 Phytopharmacological Review of Major Active Constituents

1) Curcuminoid:

It has been well reported in literature curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) inhibit TNF-induced NF-kB activation. Curcumin modulate the inflammatory response by down regulating the activity of cyclooxygenase-2 (COX-2), lipoxigenase, and inducible nitric oxide synthase (iNOS) enzymes. Inhibitory to the production of the inflammatory cytokines, tumor necrosis factor alpha (TNF-α), interleukin 1, 2, 6, 8, and 12 monocyte chemo attractant proteins (MCP), and migration
inhibitory proteins; and down regulates mitogens activated and Janus kinases. COX-2, and iNOS inhibition are likely accomplished via curcumin suppress of nuclear factor kappa B (NF-kB) activation, is involved in inflammation, proliferation and transformation\textsuperscript{22,97-98}. 

2) Steroids:
Steroids block allergic reactions and reduce the symptoms of itching, swelling and redness of skin and synthesis of certain enzymes that reduce inflammation and also suppress immune system\textsuperscript{23,99}

3) Terpenoids:
One of the most effective families of natural products is terpenoids for their medicinal value. They have been used as a anti-inflammatory to reduce the production of prostaglandin (PGE\textsubscript{2}) and also suppress the NF-kb and iNOS\textsuperscript{24,100-103}.

3.3 Literature Related to Formulations of Curcuma zedoaria Rosc
The system of ayurveda embraces within its fold drugs of plant, animal and mineral origin, both single drugs and formulations. In ancient times, ayurvedic medicines used to be prepared by the practicing physician himself for the use of patients. At this stage, the need for proper formulation development for ayurvedic medicines was felt to produce a consistent quality product. Here, reviwed C. zedoaria for its phytopharmaceutics development as ayurvedic formulations\textsuperscript{104}. Few marketed herbal formulations prepared by use of C. zedoaria such as: (I) Divya Chyawanprash with Saffron (Baba Ramdev Medicines)\textsuperscript{105} (II) Himalaya Ayurvedic Gentle Face Wash Cream\textsuperscript{106}.

Ayurvedic formulations:
Karpur\textsuperscript{\textdegree}dyarka, Rasa\textsuperscript{14}. 
3.4 Stability Study of Herbal drug

According to ICMR guideline traditional remedies have short life. To increase their stability and shelf life, and control their batch to batch variation could be challenging task for modern scientists\textsuperscript{107-109}. 