Chapter II

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The use of complementary and alternative medicines has recently increased, thereby enhancing the market for herbal products worldwide (Zollman and Vickers, 1999; Bodeker and Kronenberg, 2002). From ancient to modern times, herbs and other plants have been used as medicinal agents, first only on a folkloric basis and later developed on a scientific basis into single agent drugs. The drug discovery and development use plants as an essential route to new pharmaceutical leads (Newmann et al., 2003). The three main research approaches are (a) bioactivity- or mechanism of action-directed isolation and characterization of active compounds, (b) rational drug design-based modification and analogue synthesis, and (c) mechanism of action studies. Drug discovery is an iterative process of lead discovery (i.e., isolation of bioactive lead compound(s) from these natural sources) coupled with lead improvement (rational design and synthesis of new analogues to improve pharmacological profiles). After selection of a new lead, drug development continues outside of the academic laboratories through preclinical studies (toxicology, formulation and production) followed by clinical trials.

In the academic laboratory, drug design/structure modification employs several tools to identify the optimum chemotherapeutic agent: (a) structure-activity relationship (SAR) studies including both qualitative and quantitative SAR, (b) mechanism of action studies including drug receptor interactions and specific enzyme inhibitions, (c) drug metabolism studies including identification of bioactive metabolites and blocking of metabolic inactivation, (d) molecular modeling studies including determination of 3D pharmacophores, (e) combinatorial chemistry, including creation of peptide and nonpeptide libraries to generate new leads (Kuo-Hsiung, 2004).
Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-inflammatory and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong Shu, 1998). The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and Hostettmann, 1991). In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Williamson *et al.*, 1996).

The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries (OMS, 1991; Vulto and Smet, 1988). Eastern countries, such as China and India, have a well-established herbal medicines industry and Latin American countries have been investing in research programs in medicinal plants and the standardisation and regulation of phytomedicinal products, following the example of European countries, such as France and Germany. In Germany, 50% of phytomedicinal products are sold on medical prescription, the cost being refunded by health insurance (Gruenwald, 1997). Research into, and development of therapeutic materials from plant origin is a hard and expensive task (Borris, 1996; Turner, 1996; Williamson *et al.*, 1996). Each new drug requires an investment of around US$ 100–360 million.
and a minimum of 10 years of work, with only 1 in 10,000 tested compounds being considered promising and only 1 in 4 of these being approved as a new drug.

The drug discovery process is multi-disciplinary (De Pasquale, 1984; Verpoorte, 1989). The basic sciences involved are botany, chemistry and pharmacology, bioinformatics, including toxicology. Any research into pharmacological active natural compounds depends on the integration of these sciences. The way they are integrated and the extent of integration depend on the objectives of the study. In any case, a particular discipline should not be seen as secondary to another; quite the opposite, as each step must be carried out considering the theoretical and technical background of each of the sciences involved, otherwise the results may not be robust enough and may lead to breakdown of the process. In addition, pharmaceutical technology is fundamental to the development of any drug, including drugs of plant origin (Petrovick, 1997; Sharapin, 1997).

2.1 Kirganelia reticulata

The approach for drug development from plant resources depends on a particular aim. Different strategies will result in an herbal medicine or in an isolated active compound. However, apart from this consideration, the selection of a suitable plant for a pharmacological study is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria (Ferry and Baltassat-Millet, 1977; Soejarto, 1996; Williamson et al., 1996). The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; this is known as
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ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method. The formulation used will provide information about pharmacological activity, oral versus non-oral intake and the doses to be tested. However, certain considerations must be taken into account when the ethnopharmacological approach of plant selection is chosen.

Sensible use of these resources must be based on the amounts available, ease of access, the possibility of preservation and replanting and the establishment of priorities in relation to a desirable pharmacological activity. If possible, consideration should be given to the use of cultivated plants, which allows the production of homogeneous material, thus guaranteeing chemical homogeneity, and the use of plants from genetic enhancement projects, which preserve species threatened with extinction (Labadie, 1986). The largest research fields, as defined by the number of publications describing bioactive plant-derived compounds in the last few years are anti-tumour drugs, antibiotics, drugs active against tropical diseases, contraceptive drugs, anti-inflammatory drugs, immunomodulators, kidney protectors and drugs for psychiatric use (Hamburger and Hostettman, 1991). In general, a plant extract contains low concentrations of active compounds and a large number of promising compounds, requiring the use of sensitive bioassays suitable for the wide chemical variety and small amounts of the tested samples. Tests must be simple, reproducible, fast and cheap (Souza Brito, 1996; Brito and Nunes, 1997). Furthermore, new techniques that can fulfil different needs and be adjusted to the classical pharmacological study of natural compounds should be sought (Rates, 2001).
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After verifying the purity of an isolated active compound, the structure is determined by spectroscopic methods (Verpoorte, 1989). Once the chemical structure is defined, total or partial synthesis and preparation of derivatives and/or analogues can be considered, and modulation of the biological activity and definition of the structure–activity relationship can be carried out. After completing all these steps, large-scale isolation or partial or total synthesis is required for pharmacological evaluation in pre-clinical, clinical and toxicological trials aimed at future therapeutic use (Hamburger and Hostettman, 1991; Borris, 1996).

The phytochemical screening of the leaf extracts of *Kirganelia reticulata* revealed the presence of terpenoids, glycosides, protein, carbohydrates and absence of alkaloids and steroids by Kumar et al., (2008). Aqueous extracts of *Securinega virosa*, *Phyllanthus reticulatus* and *Breynia retusa* were screened for hepatoprotective properties against carbon tetra-chloride induced liver damage in Wistar albino rats (De Britto et al., 2005). The plant *Phyllanthus reticulatus* is claimed to have antidiabetic activity in tribal area; to validate the tribal claim, the petroleum ether and ethanolic extracts of leaves were orally tested for hypoglycemic effect in alloxan induces diabetic mice by Kumar et al., (2008). Leaves of *K. reticulata* were extracted and tested for *in vitro* antiplasmodial activity against chloroquine-sensitive (K67) and chloroquine-resistant (ENT36) strains of *Plasmodium falciparum*, where in extracts were found to be very active; and preliminary phytochemical analysis of these plants revealed the presence of different classes of primary and secondary metabolites (Omulokoli et al., 1997). RAPD markers specific for these species were identified and primers for highly specific sequence-characterized-amplified-regions (SCAR) are designed from nucleotide sequences of specific RAPD markers; which is rapid and highly specific when tested against DNA of several closely related species (Piyada et al., 2008). A
number of species of the genus *Phyllanthus* (Euphorbiaceae) have been tested for their efficacy as antivirals, partly on the basis of references to traditional usage for the treatment of diseases possibly having a viral origin. Consideration of the data from ethnobotany, *in vitro* assays and clinical trials supported the presence of some type of biological activity(s) particularly within the subgenus *Phyllanthus*. Although the herbaceous species of subgenus *Phyllanthus* have been extensively used to treat jaundice, and have generally inhibited hepadnavirus DNAp, effects on chronic infection with hepatitis B virus (HBV) or related viruses are termed negative. Other medical categories suggested possible leads for research, or possibly, herbal or galenic remedies with bona fide effects (David *et al.*, 1995).

Eight compounds, including two flavonoid glycosides, were isolated from the butanol-soluble fraction of the methanolic extract of the leaves of *Phyllanthus reticulatus* by conventional methods. A polyphenol-rich fraction, obtained by Sephadex LH-20 fractionation, was also studied using an HPLC-SPE-NMR technique leading to the characterization of six compounds including three additional flavonoid glycosides. β-Sitosterol-3-O-β-glucoside, stigmasterol-3-O-β-glucoside, methyl gallate, ellagic acid, corilagin, methyl brevifolincarboxylate, rutin (quercetin 3-rutinoside), quercetin 3-O-β-D-glucopyranoside (isoquercitrin), 2,7-di-O-methylellagic acid, rutin, isoquercitrin, kaempferol, 3-O-α-L-rhamnopyranosyl-(6→1)-β-D-glucopyranoside (kaempferol 3-rutinoside), astragalin (kaempferol 3-O-β-D-glucopyranoside), quercetin 3-O-α-L-rhamnopyranoside (quercitrin) are the characterized known polar compounds from this plant (Sio-Hong *et al.*, 2007). Ethyl acetate extract of dried root bark of this plant revealed the presence of four compounds viz. lupenone, stigmasterol, sitosterol and bergenin (Jamal *et al.*, 2009). The separations of the chemical components were carried out on leaves
using different chromatographic techniques and compounds were isolated and identified as lupeol acetate, stigmasterol and lupeol (Jamal et al., 2008). Hui et al., (1976) has reported friedelin, sitosterol, friedelan-38-02 glochidonol, 21a-hydroxyfriedelan3-one, 21a-hydroxyfriedel-4(23)-en-3-one and betulinic acid from _K. reticulata_. The lethal dose of the plant was found out by Mohammad et al., (2008). Scopoletin was isolated from the chloroform soluble fraction of a methanol extract of the stem bark of _P. reticulatus_ (Taslima et al., 2006).

The petroleum ether, carbon tetrachloride and chloroform soluble fractions of methanol extract were subjected to antimicrobial screening and brine shrimp lethality bioassay by Taslima et al., (2006). All of the partitionates showed moderate to strong inhibitory activity to microbial growth while the chloroform soluble fraction showed strongest cytotoxicity. The petroleum ether, ethyl acetate, and methanol extract of _P. reticulatus_ Poir. (Euphorbiaceae) were chosen for acetic acid-induced writhing test and carrageenan-induced rat paw edema model for evaluation of analgesic and anti-inflammatory properties. In the acetic acid-induced writhing test, the ethyl acetate extract promising inhibition of writhing, significant elongation of tail-flick time was evident both in the ethyl acetate and the methanol extracts and in carrageenan-induced rat paw edema model, the methanol extract showed inhibition of edema at the end of 4 h. These results demonstrated that the extracts possess significant analgesic and anti-inflammatory properties (Achinta et al., 2007). To validate the tribal claim of _K. reticulata_ having antidiabetic activity, the petroleum ether and ethanolic extracts of leaves of the _P. reticulatus_ were orally tested for hypoglycemic effect in alloxan induced diabetic mice. The results indicated that presence of active principles in the petroleum ether and ethanolic extracts exhibited weak hypoglycemic activity and sustained decrease in blood glucose level (Kumar et al.,
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2008). Free radicals are implicated for many diseases including Diabetes mellitus, arthritis, cancer, ageing. etc. In treatment of these diseases, antioxidant therapy has gained utmost importance. Keeping in view of the antioxidant activity, the plant was screened for in vitro antioxidant activity using different models viz. DPPH radical scavenging, ABTS radical scavenging, iron chelating activity and lipid peroxidation assay, nitric oxide scavenging assay, alkaline DMSO assay, total antioxidant capacity and non-enzymatic haemoglobin glycosylation assay by Aswatha Ram et al., (2008). In all the testing, a significant correlation was existed between concentrations of the extract and percentage inhibition of free radicals, metal chelation or inhibition of lipid peroxidation. These results clearly indicated that *P. reticulatus* is effective against free radical mediated diseases.

The various parts of plant was extracted and tested for in vitro antiplasmodial activity against chloroquine-sensitive (K67) and chloroquine-resistant (ENT36) strains of *Plasmodium falciparum* by Omulokali et al., (1997). The root, leaf and stem extracts of *Kirganelia* showed significant activity against both sensitive and resistant strain of *Plasmodium*, and hence extracts of plant used by traditional medicine have potential in the search for new and selective agents for the treatment of malaria. Two partially purified organic fractions designated as PR1 and PR2 of the fat free ethanol (95%) extract of aerial parts of *P. reticulatus* were tested for the hepatoprotective activity in rats against CCl₄-induced liver damage by Biplab et al., (2008). The rats which received the fractions showed promising hepatoprotective activity as evident from significant changes of pentobarbital-induced sleeping time, changes in serum levels of sGPT, sGOT, sALP and bilirubin and also from histopathological changes as compared to CCl₄-intoxicated rats. Out of two semi-purified organic fractions PR1 and PR2 against CCl₄-induced
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liver damage which was evident from the changes of functional, biochemical and histopathological parameters. But the prominent hepatoprotective activity was shown by PR2 as compared to PR1.

The methanolic extract of the entire plant was studied for its in vitro free radical scavenging activity in different methods viz DPPH radical scavenging assay, ABTS radical scavenging assay, O-phenanthroline assay, lipid peroxidation assay, nitric oxide scavenging assay, superoxide scavenging assay, total antioxidant and non-enzymatic haemoglobin glycosylation assay by Aswatha Ram et al., (2008). P. reticulatus extract exhibited its antioxidant action in several ways; removal of oxygen, scavenging of reactive oxygen species and nitrogen species or their precursors, inhibiting reactive oxygen species and reactive nitrogen species, binding metal ions needed for catalysis of reactive oxygen generation and up regulation of endogenous antioxidant defences. Antioxidant potential of P. reticulatus observed in the study was attributed to the presence of methanol extractable phenolic compounds and other constituents responsible for it. The cytotoxic activity of the methanolic extracts of several plant species, including traditionally used plants of Bangladesh was evaluated by the brine shrimp lethality bioassay technique. Among these, P. reticulatus plant extracts exhibited significant toxicity to brine shrimps with LC50 less than 10 µg/ml. This indicated that the plant contain potential bioactive compounds, which if properly and extensively studied, could provide many chemically interesting and biologically active drug candidates, including some with potential antitumor and antiproliferative properties. This concluded that, a thorough chemical study is required to isolate the molecules that are responsible for the activities (Mohammad et al., 2008).
2.2 Arthritis

Osteoarthritis (OA) is characterized by a loss of articular cartilage that, unlike the joint erosion of rheumatoid arthritis (RA), appears to originate within the cartilage itself from changes in chondrocyte metabolism (Byer et al., 1983; Herman et al., 1989). Within the healthy joint, chondrocytes are embedded in the cartilage matrix, where there is a complex balancing act among degradative enzymes, regulatory cytokines and their corresponding inhibitors (Hess, 1990). Unchecked damage to chondrocytes and cartilage matrix, resulting from either genetic susceptibility or biomechanical injury, leads to progressive destruction of diarthrodial joints (Mankin et al., 1986; Dieppe, 1987; Hamerman 1989; Pelletier and Howell, 1993; Williams and Jimenez, 1993). In OA, degradative enzymes are produced either directly as a result of biomechanical damage to cartilage or indirectly through simulation by cytokines or other mediators. When the chondrocyte repair capacity cannot keep up with concomitant proteoglycan depletion, cartilage loss progresses (Pelletier et al., 1983; Howell, 1986). Subsequently, this imbalance in cartilage degradation and repair impinges on the catabolic and anabolic mechanisms that maintain homeostasis in synovium and bone. Eventually, the progressive degeneration of articular cartilage is accompanied by a thickening of the subchondral bone, the formation of marginal osteophytes and involvement of other joint structures such as the synovial membrane (Pelletier et al., 1983; Howell, 1986; Sipe et al., 1994). Although OA is classified as noninflammatory synovitis, in many cases synovial membranes and synovial fluid show evidence of mild to moderate inflammation i.e elevated white cell count in synovial fluid and morphological changes to the synovial membrane (Goldenberg et al., 1982; Cohen and Goldenberg, 1985).
The enzymatic breakdown of cartilage is a key feature of OA disease progression. In response to biomechanical failure, chondrocytes release degradative enzymes that cannot be kept in check by repair processes. As the disease progresses, other joint structures such as the synovial membrane, a rich source of IL-1, IL-6 and TNF become involved. OA can be viewed as a disease in which the early, local phase involving cytokine production occurs slowly and in which the later systemic acute phase response is less pronounced than that occurs in RA (Jean, 1995). Arthritis affects more than 1.3 million adults (Helmick et al., 2008). It commonly leads to significant disability and compromises quality of life. Pharmacological treatments for arthritis target the inflammatory process by suppressing the host reaction. Despite the number of effective pharmacological agents available today, a substantial proportion of patients will experience persistent, low-level disease activity (Sesin and Bingham, 2005). This underscores the need for adjunctive therapies that are safe and can help relieve the painful symptoms of arthritis.

Synovial fluid (SF) is a dynamic reservoir for proteins originating from serum, synovial tissue, and cartilage. The composition of the SF proteome may reflect the pathophysiological conditions affecting the circulatory system and cartilage. Reliable protein markers for osteoarthritis (OA) in SF was first evaluated on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) as a function of protein loading, pH range for isoelectric focusing and concentration of acrylamide in SDS-PAGE by Hiroshi et al., (2003). These results showed that 2D-PAGE can be used under standard conditions to screen SF samples and identify a small subset of proteins in SF that are potential markers associated with OA. Despite many research efforts in recent decades, the major pathogenetic mechanisms of osteoarthritis (OA), including gene alterations occurring during OA cartilage degeneration, are poorly
understood, and there is no disease-modifying treatment approach. A study was therefore initiated in order to identify differentially expressed disease-related genes and potential therapeutic targets by Thomas et al., 2006. Many differentially expressed genes were identified, including the expected up-regulation of anabolic and catabolic matrix genes. In particular, the down-regulation of important oxidative defense genes, i.e., the genes for superoxide dismutases 2 and 3 and glutathione peroxidase 3, was prominent. This indicated that continuous oxidative stress to the cells and the matrix is one major underlying pathogenetic mechanism in OA. Also, genes that are involved in the phenotypic stability of cells, a feature that is greatly reduced in OA cartilage, appeared to be suppressed. Hence, the findings provided a reference data set on gene alterations in OA cartilage and, importantly, indicate major mechanisms underlying central cell biologic alterations that occur during the OA disease process. These results identify molecular targets that can be further investigated in the search for therapeutic interventions.

The development of increasingly high-throughput and sensitive mass spectroscopy-based proteomic techniques provides new opportunities to examine the physiology and pathophysiology of many biologic fluids and tissues. The study was conducted to determine protein expression profiles of high-abundance synovial fluid (SF) proteins in health and in the prevalent joint disease osteoarthritis (OA). Cross-sectional study of 62 patients were done, where SF proteins were separated by using one-dimensional PAGE, and the in-gel digested proteins were analyzed by electrospray ionization tandem mass spectrometry. A total of 362 spots were examined and 135 high-abundance SF proteins were identified as being expressed across all study cohorts. A total of 135 SF proteins were identified. Two subsets of OA that were not dependent on disease duration were identified using unsupervised analysis of the data. Several
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novel SF proteins were also identified. The analyses demonstrated no
disease duration-dependent differences in abundant protein composition
of SF in OA, and clearly identified two previously unappreciated yet
distinct subsets of protein profiles in this disease cohort. Additionally,
findings revealed novel abundant protein species in healthy SF whose
functional contribution to SF physiology was not previously recognized.
Finally, studies identified candidate biomarkers for OA with potential for
use as highly sensitive and specific tests for diagnostic purposes or for
evaluating therapeutic response (Reuben et al., 2007). IL-23 is the main
inductor in Th17 polarization of naive T cells, inducing IL-17 production.
IL-17 has been demonstrated to be elevated in ankylosing spondylitis
(AS). The p40 subunit is common to IL-12 and IL-23. The serum and
synovial levels of p40 IL12/23 in spondyloarthropathy (SpA) patients and
the evolution under anti-TNF was assessed by Daniel et al., (2009). The
results suggested that serum levels of p40 IL-12/23 may not be
considered as a biologic tool of disease activity assessment in SpA
patients.

Proinflammatory cytokines are mediators of inflammatory state and
cartilage degradation in both rheumatoid arthritis and osteoarthritis
(OA). In particular, interleukin (IL)-1band tumour necrosis factor-a (TNF-
a) activate chondrocytes to produce matrix-degrading factors and
promote a catabolic condition (Goldring, 2000). These cytokines up-
regulate inducible enzymes, such as nitric oxide synthase-2 (NOS-2) and
cyclo-oxygenase-2(COX-2) which is prominently expressed in the
synovium, fibrocartilage of osteophytes, and in the blood vessels in the
OA knee joint (Koki et al., 2002). Heme oxygenase-1 (HO-1) is a stress-
responsive protein with cytoprotective and antiinflammatory properties
(Alcaraz et al., 2003). The role of this enzyme in chronic inflammatory
disorders is major and this has to be established. In this regard, study
was done to authenticate function of HO-1 expressed in human OA chondrocytes can be modulated by cytokines (Fernandez, 2003). ITB which is a novel inhibitor of cyclo-oxygenase-2 (COX-2) with antiinflammatory activity was investigated for its effect on the production of catabolic or antiinflammatory mediators in osteoarthritis (OA) cartilage. In OA cartilage explants, ITB inhibited the production of prostaglandin E2 (PGE2), tumour necrosis factor-a (TNF-a) and matrix metalloproteinase-13 (MMP-13) in a concentration-dependent manner, whereas nitrite was partially reduced. On the contrary, ITB increased the production of interleukin (IL)-10 and the expression of heme oxygenase-1 (HO-1). ITB inhibited the production of catabolic mediators at concentrations able to increase IL-10 and HO-1 in OA cartilage, suggesting that this compound may be useful in the prevention of cartilage degradation (Patricia et al., 2004).

Conventional nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with a spectrum of toxic effects, notably gastrointestinal (GI) effects, because of inhibition of cyclooxygenase (COX)-1. Whether COX-2-specific inhibitors are associated with fewer clinical GI toxic effects is unknown. To determine whether celecoxib, a COX-2-specific inhibitor, is associated with a lower incidence of significant upper GI toxic effects and other adverse effects was compared with conventional NSAIDs. Incidence of prospectively defined symptomatic upper GI ulcers and ulcer complications (bleeding, perforation, and obstruction) and other adverse effects during the 6-month treatment period. In the study, celecoxib, at dosages greater than those indicated clinically, was associated with a lower incidence of symptomatic ulcers and ulcer complications combined, as well as other clinically important toxic effects, and compared with NSAIDs at standard dosages. The decrease in upper GI toxicity was
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strongest among patients not taking aspirin concomitantly (Fred et al., 2000).

Lactic acid-producing bacteria (LAB) probiotics demonstrate immunomodulating and anti-inflammatory effects and the ability to lessen the symptoms of arthritis in both animals and humans. Clinical pilot trial was conducted to evaluate the effects of the LAB probiotic preparation, *Bacillus coagulans* GBI-30, 6086, on symptoms and measures of functional capacity in patients with rheumatoid arthritis in combination with pharmacological anti-arthritic medications. *Bacillus coagulans* GBI-30, 6086 treatment resulted in greater improvement in patient global assessment and self-assessed disability; reduction in CRP; as well as the ability to walk 2 miles, reach, and participate in daily activities. There were no treatment related adverse events reported throughout the study. Results of this pilot study suggested that adjunctive treatment with *Bacillus coagulans* GBI-30, 6086 LAB probiotic appears to be a safe and effective for patients suffering from arthritis (David et al., 2010).

Arthritis is a prevalent and debilitating disease that affects the joints. Infiltration of blood-derived cells in the affected joints upon activation generates reactive oxygen/nitrogen species, resulting in an oxidative stress. One approach to counteract this oxidative stress is the use of antioxidants as therapeutic agents. The methanolic extract of *Albizia lebbeck* (AL) which exhibits significant anti-inflammatory activity, was evaluated for the possible mode of action by studying its antioxidant potential in adjuvant-induced arthritic rats by Nimish et al., (2010). On 21st day of experiment; the biological estimation and radiological observation were carried out along with rheumatoid factor and arthritic index. Study concluded that *Albizia lebbeck* methanolic extract possesses
strong anti-arthritis and anti-oxidant property. Adjuvant arthritis is one of the extensively used models of chronic inflammatory joint disorder such as rheumatoid arthritis. The research was designed to examine the antioxidative effect of *Gaultheria fragrantissima* Wall. (Ericaceae) against complete Freund’s adjuvant induced arthritis. The protective effect was evaluated by DPPH radical scavenging activity, alterations in paw volume, lipid peroxidation (measured in terms of MDA), antioxidant, enzymatic (SOD, CAT, GPx, GR and GST), nonenzymatic (GSH), and marker enzymes (AST, ALT, ALP and GGT) levels from liver and serum of adjuvant induced and treatment groups. The biochemical alterations were significantly ameliorated after administration of *Gaultheria fragrantissima* leaf extract to arthritic animals (Shanmugarajan *et al.*, 2009).

Turmeric has been used for centuries in Ayurvedic medicine as a treatment for inflammatory disorders including arthritis. Based on this traditional usage, dietary supplements containing turmeric rhizome and turmeric extracts are also being used in the western world for arthritis treatment and prevention. Therefore, the studies were undertaken to determine the *in vivo* efficacy of well characterized curcuminoid-containing turmeric extracts in the prevention or treatment of arthritis using streptococcal cell wall (SCW) induced arthritis, a well-described animal model of arthritis. Arthritic index, a clinical measure of joint swelling, was used as the primary endpoint for assessing the effect of extracts on joint inflammation. In conclusion, the data documented the *in vivo* anti-arthritis efficacy of an essential oil depleted turmeric fraction and suggested that the three major curcuminoids are responsible for the anti-arthritis effect (Janet *et al.*, 2006). Quercetin is one of common flavonols biosynthesized by plants and has been suggested to modulate inflammatory responses in various models. The study investigated *in vivo*
effects of oral or intra-cutaneous Quercetin in chronic rat adjuvant-induced arthritis was carried out by Maria et al., (2006). Growth delay and arthritic scores were evaluated daily after induction in Lewis rats. Anti-arthritic effects produced were correlated with significant decrease of inflammatory mediators produced by peritoneal macrophages, *ex vivo* and *in vitro*. The data indicated that Quercetin is a potential anti-inflammatory therapeutic and preventive agent targeting the inflammatory response of macrophages. Various extracts of plants such as *Paeonia lactiflora*, *Picrodita nitida*, *Bauhinia racemosa*, *Vitex negundo*, *Premna serratifolia*, *Anisomeles malabarica*, *Cyperus esculentus*, *Cyperus rotundus*, *Ajuga bracteosa*, *Capparis erythrocarpus*, *Justicia gendarussa*, *Aristolochia Bracteata*, *Aristolochia Bracteata*, *Glycyrrhiza glabra*, *Boswellia serrata* etc., have been evaluated for *in vivo* anti-arthritic activity using different experimental models. Various *in vitro* anti-arthritic pharmacological models were studied, such as inhibition of protein denaturation, effect of membrane stabilization and proteinase inhibitory action of several herbal extracts from *Abutilon indicum*, *Manilkara zapota*, *Anisomeles malabarica*, *Asystasia dalzelliana*, *Piper nigrum*, *Terminalia Chebula*, *Centella asiatica* etc.

Current therapeutic approaches to the treatment of inflammatory diseases are centered on cyclooxygenase (both COX-1 and 2) proinflammatory enzymes but present available drugs of this category are associated with undesirable gastrointestinal and cardiovascular side effects (Vane, 1971; Garner, 1992). Recent scientific advents draw out the secrets of inflammation cache and understanding the involvement of several factors acting as stimulators or inhibitors thus opening new avenues for drug discoveries. Several bio-molecules such as proinflammatory cytokines, components of signal transduction and matrix degrading enzymes resolve inflammatory responses, might be new
targets for treatment of chronic inflammatory diseases. The recent advances in drug research are focusing interleukin-1, TNF-a, p38 kinase, c-Jun N-terminal kinase MAP kinase, NFkB and matrix metalloproteinases. The biological roles of these inflammatory mediators are clearly understood thus offering new targets for design of novel inhibitors for incurable inflammatory diseases (Matsukawa et al., 1997; Mussener et al., 1997; Herlaar and Brown, 1999; Ono and Han, 2000; Woolley and Tetlow, 2000; Choy and Panayi, 2001). In treating the inflammatory diseases, NSAIDs and selective COX-2 inhibitors have been conventionally the most extensively used drugs till date. However, their long-term treatment has been demonstrated to have highly adverse side effects and it has been observed that the use of rofecoxib, selective COX-2 inhibitor might even lead to fatalities due to cardiovascular and thrombotic events. Proinflammatory cytokines and components of signal transduction play a central role in the pathology of inflammation, some proteinaceous cytokine inhibitors viz. infliximab were effective either as a monotherapy or in combination with other drugs effective in treating arthritis.

Prolonged use of these cytokine inhibitors may lead to post-treatment infections and therefore there is a quest to obtain small molecules that may inhibit these proinflammatory or intracellular signals. Further, the cost effectiveness and mode of administration of the cytokine inhibitors are not at desirable levels. Apart from these proinflammatory cytokines as a target for new antiarthritic drug discovery, the components of signal transduction like p38 kinase, JNK and NF-kappaB can be targeted. Some of small molecules that inhibit p38 kinase are in the final stages of clinical trials. The success of these inhibitors depends on how best they pass through the clinical trials for safe use in human beings. Therefore, the present research proposes that there is a paradigm shift in the drug
design and discovery attempts towards anti-inflammatory diseases. Slowly the attention is drifting towards the design, synthesis and lead optimization of inhibitors for signal transduction and anti-cytokine drugs rather than NSAIDS and COX proteins (Kulkarni et al., 2006).