1. INTRODUCTION

Nature has bestowed our planet with an enormous wealth of medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention and cure of diseases and ailments.

Plants are important and basic of preventive and curative health care system since immemorial. Disease is as old as mankind and use of indigenous herbal medicine is a very ancient art and an integral part of treatment.\(^1\) Traditional medicinal herbs have served as a potential source of alternative medicine and different healthcare products. Knowledge of herbal medicines has derived from rich traditions of ancient civilizations and scientific heritage. From ancient time Indian, Chinese, Egyptian, Greek, Roman and Syrian medicinal system documented the use of different plant based medicine for different diseases.\(^2\)

According to WHO, nearly 75-80\% of world population still depends on herbal medicines. Active constituents from plant sources directly used as therapeutic agent and phytoconstituents are also served as lead molecule for the synthesis of various drugs. Folk medicine and their use against diseases in different cultures is a vast traditional knowledge; which is based on the necessities, instinct, observation, trial and error and long experience of ancient/tribal people. Indigenous or herbal medicines confer considerable economic benefits to most rural and poor people.

WHO noted that about 25\% of modern medicines are descended from plants sources used traditionally and research on traditional medicinal herbal plant leads discovery of 75\% of herbal drugs.\(^3\)

Herbal drugs constitute a major share of all the officially recognized systems of health in India. Currently, there is no separate category of herbal drugs or dietary supplements, as per the Indian Drugs Act. However, there is a vast experiential-evidence base for many of the natural drugs. This offers immense
opportunities for Observational Therapeutics and Reverse Pharmacology. Evidence-based herbals are widely used in the diverse systems and manufactured, as per the pharmacopoeial guidelines, by a well-organised industry.

The ethnomedicinal survey can bring out many different clues for development of drugs to treat and cure diseases in human beings. Herbal medicines are assumed to be of immense significance in the primary healthcare of individuals and communities in many developing countries. People in tribal areas in the country are being developed and their lifestyle is changing rapidly as they are being absorbed in the main stream of population. In the current scenario it is an urgent need to evaluate some unique (new and less known) medicinal uses of the plants utilized by the tribals and encourage preservation of their culture, traditional knowledge, conservation and sustainable utilization of plant wealth. The detailed investigation and documentation of medicinal plants used in local health traditions and their pharmacological evaluation can lead to the development of invaluable plant drugs for many dreaded diseases. Many plant derived drugs used in modern medicine are developed by the therapeutic approach and more than 100 drugs of known structure that are extracted from higher plants are used in allopathic medicine. Phytotherapeutic approach emphasis on the development of new drugs whose extraction and fractionation have emanated on the basis of therapeutic activity. The standard fraction of an active extract or mixture of fractions may prove better therapeutically, less toxic and inexpensive compared to pure isolated compound drugs. However, crude plant preparations require modern standards of safety and efficacy. Modern bioassay methods and phytochemical profile do provide ways and means of developing quality control of crude preparations or fractions. WHO has adopted resolution that herbal medicine is of great importance to the health of individuals and communities.4-6

Inflammation is an important feature of great number of diseases. Inflammatory diseases including different types of rheumatic diseases are a
major cause of morbidity. Inflammation is a response of the tissue to an injury, infection, irritation or foreign substance. It is a part of host defence, but when the response becomes too great it may be far worse than the disease itself and in extreme conditions, it may be fatal.\textsuperscript{7-8}

Rheumatic diseases have affected mankind since ages and are one of the commonest inflammatory conditions in developing countries. Rheumatoid arthritis (RA) forms a major prototype of rheumatic diseases and is a common cause of disability.\textsuperscript{9}

**Arthritis**

Arthritis is inflammation of a joint. Symptoms of arthritis may include pain, swelling, redness, warmth and limitation of movement. There are over 100 types of arthritis. Three common types are osteo-arthritis, rheumatoid arthritis and gout.

Osteo-arthritis is a condition in which the cartilage that protects and cushions joints breaks down over time. Eventually, the bone-formerly separated by the cartilage-rub against each other, resulting in damage to the tissue and underlying bone and causing painful joint symptoms.

Gout is an inflammatory joint disease that causes acute pain and swelling. It is a form of arthritis that develops when uric acid crystals form in and around the joints, commonly affecting the big toe joint (this symptom is called podagra). People who have gout may have a very painful attack in one or two joints followed by the total disappearance of all symptoms until the next attack.

**Rheumatoid arthritis (RA)** is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process
often leads to the destruction of articular cartilage and ankylosis of the joints. Rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium, pleura, and sclera, and also nodular lesions, most common in subcutaneous tissue. Although the cause of rheumatoid arthritis is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and RA is considered a systemic autoimmune disease.

**Epidemiology**

About 1% of the world's population is affected by rheumatoid arthritis, women three times more often than men. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. It is a clinical diagnosis made on the basis of symptoms, physical examination, radiographs (X-rays) and labs, although the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) publish diagnostic guidelines. Diagnosis and long-term management are typically performed by a rheumatologist, an expert in joint, muscle and bone diseases. 10

1.1 Signs and symptoms

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1.1 Signs and symptoms

While rheumatoid arthritis primarily affects joints, problems involving other organs of the body are known to occur. Extra-articular ("outside the joints") manifestations other than anemia (which is very common) are clinically evident in about 15–25% of individuals with rheumatoid arthritis.\(^{11}\) It can be difficult to determine whether disease manifestations are directly caused by the rheumatoid process itself, or from side effects of the medications commonly used to treat it – for example, lung fibrosis from methotrexate or osteoporosis from corticosteroids.
1.1.1 Joints

Figure 1.1: A diagram showing how rheumatoid arthritis affects a joint

The arthritis of joints known as synovitis is inflammation of the synovial membrane that lines joints and tendon sheaths. Joints become swollen, tender and warm, and stiffness limits their movement. With time RA nearly always affects multiple joints (it is a polyarthritis), most commonly small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved. Synovitis can lead to tethering of tissue with loss of movement and erosion of the joint surface causing deformity and loss of function.\textsuperscript{12}
1.1.2 Skin

The *rheumatoid nodule*, which is often subcutaneous, is the cutaneous feature most characteristic of rheumatoid arthritis. The initial pathologic process in nodule formation is unknown but may be essentially the same as the synovitis, since similar structural features occur in both. The nodule has a central area of fibrinoid necrosis that may be fissured and which corresponds to the fibrin-rich necrotic material found in and around an affected synovial space. Surrounding the necrosis is a layer of palisading macrophages and fibroblasts, corresponding to the intimal layer in synovium and a cuff of connective tissue containing clusters of lymphocytes and plasma cells, corresponding to the subintimal zone in synovitis. The typical rheumatoid nodule may be a few millimetres to a few centimetres in diameter and is usually found over bony prominences, such as the olecranon, the calcaneal tuberosity, the metacarpophalangeal joint, or other areas that sustain repeated mechanical stress. Nodules are associated with a positive RF (rheumatoid factor) titer and severe erosive arthritis. Rarely, these can occur in internal organs or at diverse sites on the body.

Several forms of *vasculitis* occur in rheumatoid arthritis. A benign form occurs as microinfarcts around the nailfolds. More severe forms include livedo reticularis, which is a network (reticulum) of erythematous to purplish discoloration of the skin caused by the presence of an obliterative cutaneous capillaropathy.

1.1.3 Lungs

Fibrosis of the lungs is a recognized response to rheumatoid disease. It is also a rare but well recognized consequence of therapy (for example with methotrexate and leflunomide). Caplan’s syndrome describes lung nodules in individuals with rheumatoid arthritis and additional exposure to coal dust.
Pleural effusions are also associated with rheumatoid arthritis. Another complication of RA is Rheumatoid Lung disease.\textsuperscript{13}

\subsection*{1.1.4 Kidneys}

Renal amyloidosis can occur as a consequence of chronic inflammation.\textsuperscript{14} Rheumatoid arthritis may affect the kidney glomerulus directly through a vasculopathy or a mesangial infiltrate but this is less well documented (though this is not surprising, considering immune complex-mediated hypersensitivities are known for pathogenic deposition of immune complexes in organs where blood is filtered at high pressure to form other fluids, such as urine and synovial fluid\textsuperscript{15}). Treatment with Penicillamine and gold salts are recognized causes of membranous nephropathy.

\subsection*{1.1.5 Heart and blood vessels}

People with rheumatoid arthritis are more prone to atherosclerosis, and risk of myocardial infarction (heart attack) and stroke is markedly increased.\textsuperscript{16,17} Other possible complications that may arise include: pericarditis, endocarditis, left ventricular failure, valvulitis and fibrosis.\textsuperscript{18} Many people with rheumatoid arthritis do not experience the same chest pain that others feel when they have angina or myocardial infarction. To reduce cardiovascular risk, it is crucial to maintain optimal control of the inflammation caused by rheumatoid arthritis (which may be involved in causing the cardiovascular risk), and to use exercise and medications appropriately to reduce other cardiovascular risk factors such as blood lipids and blood pressure.

\subsection*{1.1.6 Other}

Ocular

The eye is directly affected in the form of episcleritis which when severe can very rarely progress to perforating scleromalacia. Rather more common is the indirect effect of keratoconjunctivitis sicca, which is a dryness of eyes and
mouth caused by lymphocyte infiltration of lacrimal and salivary glands. When severe, dryness of the cornea can lead to keratitis and loss of vision. Preventive treatment of severe dryness with measures such as nasolacrimal duct occlusion is important.

Hepatic

Cytokine production in joints and/or hepatic Kupffer cells leads to increased activity of hepatocytes with increased production of acute-phase proteins, such as C-reactive protein and increased release of enzymes such as alkaline phosphatase into the blood. In Felty's syndrome, Kupffer cell activation is so marked that the resulting increase in hepatocyte activity is associated with nodular hyperplasia of the liver, which may be palpably enlarged. Although Kupffer cells are within the hepatic parenchyma, they are separate from hepatocytes. As a result there is little or no microscopic evidence of hepatitis (immune-mediated destruction of hepatocytes). Hepatic involvement in RA is essentially asymptomatic.

Hematological

Anemia is by far the most common abnormality of the blood cells. Rheumatoid arthritis may cause a warm autoimmune hemolytic anemia. The red cells are of normal size and colour (normocytic and normochromic). A low white blood cell count (neutropenia) usually only occurs in patients with Felty's syndrome with an enlarged liver and spleen. The mechanism of neutropenia is complex. An increased platelet count (thrombocytosis) occurs when inflammation is uncontrolled, as does the anemia.

Neurological

Peripheral neuropathy and mononeuritis multiplex may occur. The most common problem is carpal tunnel syndrome caused by compression of the median nerve by swelling around the wrist. Atlanto-axial subluxation can
occur, owing to erosion of the odontoid process and/or transverse ligaments in the cervical spine's connection to the skull. Such an erosion (>3mm) can give rise to vertebrae slipping over one another and compressing the spinal cord. Clumsiness is initially experienced, but without due care this can progress to quadriplegia.

Constitutional symptoms

Constitutional symptoms including fatigue, low grade fever, malaise, morning stiffness, loss of appetite and loss of weight are common systemic manifestations seen in patients with active rheumatoid arthritis.

Osteoporosis

Local osteoporosis occurs in RA around inflamed joints. It is postulated to be partially caused by inflammatory cytokines. More general osteoporosis is probably contributed to by immobility, systemic cytokine effects, local cytokine release in bone marrow and corticosteroid therapy.

Lymphoma

The incidence of lymphoma is increased in RA, although it is still uncommon.\textsuperscript{20,21}
1.2 Diagnosis

1.2.1 Imaging

**Figure 1.2:** X-ray of the hand in rheumatoid arthritis

X-rays of the hands and feet are generally performed in people with a polyarthritis. In rheumatoid arthritis, there may be no changes in the early stages of the disease, or the x-ray may demonstrate juxta-articular osteopenia, soft tissue swelling and loss of joint space. As the disease advances, there may be bony erosions and subluxation. X-rays of other joints may be taken if symptoms of pain or swelling occur in those joints.

Other medical imaging techniques such as magnetic resonance imaging (MRI) and ultrasound are also used in rheumatoid arthritis.

1.2.2 Blood tests

When RA is clinically suspected, immunological studies are required, such as testing for the presence of rheumatoid factor (RF, a non-specific antibody). A negative RF does not rule out RA; rather, the arthritis is called *seronegative*. This is the case in about 15% of patients. During the first year of illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time. RF is also seen in other illnesses, for example Sjögren's syndrome, Hepatitis C, chronic infections and in
approximately 10% of the healthy population, therefore the test is not very specific.

Because of this low specificity, new serological tests have been developed, which test for the presence of the anti-citrullinated protein antibodies (ACPAs) or anti-CCP. Like RF, these tests are positive in only a proportion (67%) of all RA cases, but are rarely positive if RA is not present, giving it a specificity of around 95%. As with RF, there is evidence for ACPAs being present in many cases even before onset of clinical disease.

The most common tests for ACPAs are the anti-CCP (cyclic citrullinated peptide) test and the Anti-MCV assay (antibodies against mutated citrullinated Vimentin). Recently a serological point-of-care test (POCT) for the early detection of RA has been developed. This assay combines the detection of rheumatoid factor and anti-MCV for diagnosis of rheumatoid arthritis and shows a sensitivity of 72% and specificity of 99.7%.24,25

Also, several other blood tests are usually done to allow for other causes of arthritis, such as erythrocyte sedimentation rate (ESR), C-reactive protein, full blood count, renal function, liver enzymes and other immunological tests (e.g., antinuclear antibody/ANA) are all performed at this stage.

1.2.3 Criteria

In 2010 the 2010 ACR / EULAR Rheumatoid Arthritis Classification Criteria were introduced.26 The classification criteria, jointly published by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) establish a point value between 0 and 10. Every patient with a point total of 6 or higher is unequivocally classified as an RA patient, provided he has synovitis in at least one joint and given that there is no other diagnosis better explaining the synovitis. Four areas are covered in the diagnosis.
joint involvement, designating the metacarpophalangeal joints, proximal interphalangeal joints, the interphalangeal joint of the thumb, second through third metatarsophalangeal joint and wrist as small joints, and elbows, hip joints and knees as large joints:

- Involvement of 1 large joint gives 0 points
- Involvement of 2-10 large joints gives 1 point
- Involvement of 1-3 small joints (with or without involvement of large joints) gives 2 points
- Involvement of 4-10 small joints (with or without involvement of large joints) gives 3 points
- Involvement of more than 10 joints (with involvement of at least 1 small joint) gives 5 points

- acute phase reactants: 1 point for elevated erythrocyte sedimentation rate, ESR, or elevated CRP value (c-reactive protein)
- duration of arthritis: 1 point for symptoms lasting six weeks or longer

The new criteria accommodate to the growing understanding of rheumatoid arthritis and the improvements in diagnosing RA and disease treatment. 27

The criteria are not intended for the diagnosis for routine clinical care; they were primarily intended to categorize research (classification criteria). In clinical practice, the following criteria apply:

- two or more swollen joints
- morning stiffness lasting more than one hour for at least six weeks
- the detection of rheumatoid factors A negative autoantibody result does not exclude a diagnosis of RA.
1.3 Pathophysiology and causes

Rheumatoid arthritis is a form of autoimmunity, the causes of which are still incompletely known. It is a systemic (whole body) disorder principally affecting synovial tissues.

The key pieces of evidence relating to pathogenesis are:

1. A genetic link with HLA-DR4 and related allotypes of MHC Class II and the T cell-associated protein PTPN22.
2. A undeniable link with cigarette smoking and the pathogenesis of rheumatoid vasculitis, a typical feature of this condition.28

3. A remarkable deceleration of disease progression in many cases by blockade of the cytokine TNF (alpha).

4. A similar dramatic response in many cases to depletion of B lymphocytes, but no comparable response to depletion of T lymphocytes.

5. A more or less random pattern of whether and when predisposed individuals are affected.

6. The presence of autoantibodies to IgGFc, known as rheumatoid factors (RF), and antibodies to citrullinated peptides (ACPA).

These data suggest that the disease involves abnormal B cell–T cell interaction, with presentation of antigens by B cells to T cells via HLA-DR eliciting T cell help and consequent production of RF and ACPA. Inflammation is then driven either by B cell or T cell products stimulating release of TNF and other cytokines. The process may be facilitated by an effect of smoking on citrullination but the stochastic (random) epidemiology suggests that the rate limiting step in genesis of disease in predisposed individuals may be an inherent stochastic process within the immune response such as immunoglobulin or T cell receptor gene recombination and mutation.
If TNF release is stimulated by B cell products in the form of RF or ACPA-containing immune complexes, through activation of immunoglobulin Fc receptors, then RA can be seen as a form of Type III hypersensitivity.\textsuperscript{29,30}

There is little doubt that both B and T cells are essential to the disease. However, there is good evidence for neither cell being necessary at the site of inflammation. This tends to favour immune complexes (based on antibody synthesised elsewhere) as the initiators, even if not the sole perpetuators of inflammation.

Although TNF appears to be the dominant, other cytokines (chemical mediators) are likely to be involved in inflammation in RA. Blockade of TNF does not benefit all patients or all tissues (lung disease and nodules may get worse). Blockade of IL-1, IL-15 and IL-6 also have beneficial effects and IL-17 may be important. Constitutional symptoms such as fever, malaise, loss of appetite and weight loss are also caused by cytokines released in to the blood stream.

As with most autoimmune diseases, it is important to distinguish between the cause(s) that trigger the process, and those that may permit it to persist and progress.

1.3.1 Possible infectious triggers

It has long been suspected that certain infections could be triggers for this disease. The "mistaken identity" theory suggests that an infection triggers an immune response, leaving behind antibodies that should be specific to that organism. The antibodies are not sufficiently specific, though, and set off an immune attack against part of the host. Because the normal host molecule "looks like" a molecule on the offending organism that triggered the initial immune reaction—this phenomenon is called molecular mimicry. Some infectious organisms suspected of triggering rheumatoid arthritis include
Mycoplasma, Erysipelothrix, parovirus B19 and rubella, but these associations have never been supported in epidemiological studies.

1.3.2 Psychological factors

There is no evidence that physical and emotional effects or stress could be a trigger for the disease. The many negative findings suggest that either the trigger varies, or that it might in fact be a chance event inherent with the immune response.\textsuperscript{31-33}

1.3.3 Continued abnormal immune response

The factors that allow an abnormal immune response, once initiated, to become permanent and chronic, are becoming more clearly understood. The genetic association with HLA-DR4, as well as the newly discovered associations with the gene PTPN22 and with two additional genes,\textsuperscript{34} all implicate altered thresholds in regulation of the adaptive immune response. It has also become clear from recent studies that these genetic factors may interact with the most clearly defined environmental risk factor for rheumatoid arthritis, namely cigarette smoking.\textsuperscript{35,36} Other environmental factors also appear to modulate the risk of acquiring RA, and hormonal factors in the individual may explain some features of the disease, such as the higher occurrence in women, the not-infrequent onset after child-birth, and the (slight) modulation of disease risk by hormonal medications. Exactly how altered regulatory thresholds allow the triggering of a specific autoimmune response remains uncertain. However, one possibility is that negative feedback mechanisms that normally maintain tolerance of self are overtaken by aberrant positive feedback mechanisms for certain antigens such as IgG Fc (bound by RF) and citrullinated fibrinogen (bound by ACPA).

Once the abnormal immune response has become established (which may take several years before any symptoms occur), plasma cells derived from B lymphocytes produce rheumatoid factors and ACPA of the IgG and IgM
classes in large quantities. These are not deposited in the way that they are in systemic lupus. Rather, they appear to activate macrophages through Fc receptor and perhaps complement binding. This can contribute to inflammation of the synovium, in terms of edema, vasodilation and infiltration by activated T-cells. Synovial macrophages and dendritic cells further function as antigen presenting cells by expressing MHC class II molecules, leading to an established local immune reaction in the tissue. The disease progresses in concert with formation of granulation tissue at the edges of the synovial lining (pannus) with extensive angiogenesis and production of enzymes that cause tissue damage. Modern pharmacological treatments of RA target these mediators. Once the inflammatory reaction is established, the synovium thickens, the cartilage and the underlying bone begins to disintegrate and evidence of joint destruction accrues.

1.3.4 Role of vitamin D

The discovery of the vitamin D receptor (VDR) in the cells of the immune system and the fact that activated dendritic cells produce the vitamin D hormone suggested that vitamin D could have immunoregulatory properties. VDR, a member of the nuclear hormone receptor superfamily, was identified in mononuclear cells, dendritic cells, antigen-presenting cells, and activated T-B lymphocytes. In synthesis, the most evident effects of the D-hormone on the immune system seem to be in the downregulation of the Th1-driven autoimmunity. Low serum levels of vitamin D3 might be partially related, among other factors, to prolonged daily darkness (reduced activation of the pre vitamin D by the ultra violet B sunlight), different genetic background (i.e. vitamin D receptor polymorphism) and nutritional factors, and explain the latitude-related prevalence of autoimmune diseases such as rheumatoid arthritis (RA), by considering the potential immunosuppressive roles of vitamin D. Recently, greater intake of vitamin D was associated with a lower risk of RA, as well as a significant clinical improvement was strongly correlated with the immunomodulating potential in vitamin D-treated RA patients.
In patients with rheumatoid arthritis measuring vitamin D levels seems particularly pertinent as deficiency is highly prevalent in the group. Vitamin D is already known to be important in preventing osteoporosis and fracture falls, which are also common in RA.\textsuperscript{39}

1.4 Treatment

There is no known cure for rheumatoid arthritis, but many different types of treatment can alleviate symptoms and/or modify the disease process. Recommendations of the American College of Rheumatology (ACR), published in 2008, followed a trend in supporting earlier, more aggressive treatment of RA, and reflected heightened expectations of treatment effectiveness, including remission or substantial alleviation of symptoms for a rising percentage of patients.

ACR recommends that RA should generally be treated with at least one specific anti-rheumatic medication, also named DMARD (see below), to which other medications may be added depending on how long a person has had RA, how active the disease is, and prognostic factors (such as X-ray evidence of bone erosion; elevation of blood factors such as Rheumatoid factor, anti-cyclic citrullinated peptide, C-reactive protein, and erythrocyte sedimentation rate, age and gender, physical functioning, and smoking, for example).\textsuperscript{40}

Pharmacological treatment of RA can be divided into disease-modifying antirheumatic drugs (DMARDs), anti-inflammatory agents and analgesics. Treatment also includes rest and physical activity.\textsuperscript{41,42}

1.4.1 Disease modifying anti-rheumatic drugs (DMARDs)

The term Disease-modifying anti-rheumatic drug (DMARD) originally meant a drug that affects biological measures such as ESR and haemoglobin and autoantibody levels, but is now usually used to mean a drug that reduces the rate of damage to bone and cartilage. DMARDs have been found both to
produce durable symptomatic remissions and to delay or halt progression. This is important as such damage is usually irreversible. Anti-inflammatories and analgesics improve pain and stiffness but do not prevent joint damage or slow the disease progression.

Disease-modifying anti-rheumatic drugs have been used in the treatment of rheumatic arthritis for a long time now. Over 90% of rheumatologists now use combination therapy of multiple disease modifying drugs for rheumatoid arthritis as it has become apparent that using combination of these drugs does not increase their relative toxicity profiles. Common combinations of DMARDs include methotrexate – hydroxychloroquine, methotrexate – sulfasalazine, sulfasalazine – hydroxychloroquine, and methotrexate – hydroxychloroquine – sulfasalazine.

1.4.1a Traditional small molecular mass drugs

Chemically synthesised DMARDs:

- azathioprine
- ciclosporin (cyclosporine A)
- D-penicillamine
- gold salts
- hydroxychloroquine
- leflunomide
- methotrexate (MTX)
- minocycline
- sulfasalazine (SSZ)

1.4.1b Biological agents

Biological agents (biologics) include:

- tumor necrosis factor alpha (TNFα) blockers – etanercept, infliximab, adalimumab, certolizumab pegol, golimumab
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- Interleukin 1 (IL-1) blockers – anakinra
- monoclonal antibodies against B cells – rituximab
- T cell costimulation blocker – abatacept
- Interleukin 6 (IL-6) blockers – tocilizumab (an anti-IL-6 receptor antibody)

1.4.2 Anti-inflammatory agents and analgesics

Anti-inflammatory agents include:

- glucocorticoids
- Non-steroidal anti-inflammatory drug (NSAIDs, most also act as analgesics)

Analgesics include:

- paracetamol
- opiates
- diproqualone
- lidocaine topical

Historic treatments for RA have also included: rest, ice, compression and elevation, apple diet, nutmeg, some light exercise every now and then, nettles, bee venom, copper bracelets, rhubarb diet, extractions of teeth, fasting, honey, vitamins, insulin, magnets, and electroconvulsive therapy (ECT). Most of these have either had no effect at all, or their effects have been modest and transient, while not being generalizable.

NSAIDs used in the treatment of RA include ibuprofen, naproxen, meloxicam, etodolac, nabumetone, sulindac, tolementin, choline magnesium salicylate, diclofenac, diflusinal, indomethicin, Ketoprofen, Oxaprozin, and piroxicam.
1.4.3 Surgery

In early phases of the disease, an arthroscopic or open synovectomy may be performed. It consists of the removal of the inflamed synovia and prevents a quick destruction of the affected joints. In older patients, the yttrium synovectomy may be performed. It is successful in approximately half of patients. The surgery is mostly done on knee, elbow, shoulder, ankle or tarsal joints. It has to be performed before the destruction of the cartilage. Severely affected joints may require joint replacement surgery, such as knee replacement. Postoperatively, physiotherapy is always necessary.

1.4.4 Other therapies

Regular exercise is important for maintaining joint mobility and making the joint muscles stronger. A Cochrane Review of studies determined that exercise programs designed to improve strength and stamina were safe and led to moderate benefits for RA sufferers.

Other therapies are weight loss, orthoses, occupational therapy, podiatry, physiotherapy, immunoadsorption therapy, joint injections, and special tools to improve hand movements (e.g., special tin-openers).

The incidence of RA is in the region of 3 cases per 10,000 populations per annum. Onset is uncommon under the age of 15 and from then on the incidence rises with age until the age of 80. It is up to three times more common in smokers than non-smokers, particularly in men, heavy smokers, and those who are rheumatoid factor positive.

Although several modern drugs are used to treat these types of disorders, their prolonged use may cause severe adverse side effects on chronic administration, the most common being gastrointestinal bleeding and peptic ulcers. Consequently there is a need to develop new anti-inflammatory agents with minimum side effects.
Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases.

Considering the adverse effects of synthetic drugs people are looking for natural remedies which are safe and effective.\textsuperscript{52} It is documented that 80% of the world’s population has faith in traditional medicine, particularly plant drugs for their primary healthcare.

It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries. In order to promote Indian medicinal plants, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines.\textsuperscript{53,54}

1.5 Plants used as anti-inflammatory and anti-rheumatic

Natural products have long been recognized as an important source of therapeutically effective medicines. Different approaches used to analyze the anti-inflammatory potential of plant and plant derived compounds have been developed in the past years. Despite the tremendous progress in medical research during the past decades, the treatment of many serious diseases including pain and inflammation is still problematic.\textsuperscript{55} Currently used anti-inflammatory and analgesic drugs are associated with some severe side effects; therefore there is a need for the development of potent analgesic and anti-inflammatory drugs with fewer side effects. Herbal medicine showed safety, efficacy, cultural acceptability and lesser side effects than the synthetic drugs. The number of chemical compounds, found within the plant kingdom is a part of the physiological functions of living flora and are supposed to have better compatibility with the human body.\textsuperscript{56} Different phytoconstituents like alkaloids,
flavonoids, xanthone, coumarin, sterols, withaferin-A, andrographolide etc., are also proved effective as analgesic and anti-inflammatory agent. Literature survey of anti-inflammatory and anti-rheumatic drugs is summarized in the form of table as below:-
Table 1.1: Anti-inflammatory and Anti-rheumatic plant drugs/extracts, uses and experimental models

<table>
<thead>
<tr>
<th>PLANT NAME</th>
<th>TRADITIONAL USES</th>
<th>PART USED</th>
<th>TYPE OF EXTRACT</th>
<th>EXPERIMENTAL MODELS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Albizia lebbeck,</em> Family: <em>Leguminosae</em></td>
<td>Barks and leaves are used to relief tooth ache, diseases of the gum, allergic disorders and bronchial asthma</td>
<td>Bark</td>
<td>Cold extraction of mixture of Petroleum ether, ethyl acetate and methanol</td>
<td>Acetic acid induced writhing, radiant heat tail flick method(^{58})</td>
</tr>
<tr>
<td><em>Annona squamosa,</em> Family: <em>Annonaceae</em></td>
<td>Used to stop diarrhea, dysentery and used as a cold remedy, insecticide, expectorant, tonic</td>
<td>Bark</td>
<td>Petroleum ether</td>
<td>Acetic acid induced writhing test, carrageenan induced paw oedema(^{59})</td>
</tr>
<tr>
<td><em>Artemisia absinthium,</em> Family: <em>Compositae</em></td>
<td>Used as tonic, stomachic, febrifuge, gastric pain, antihelmintic</td>
<td>Seed, stem</td>
<td>Methanol extract</td>
<td>Tail immersion method, carrageenan induced paw edema(^{60})</td>
</tr>
<tr>
<td><em>Bauhinia racemosa,</em> Family: <em>Caesalpiniaceae</em></td>
<td>Bark, root, flower used in hemorrhoids, cough, diarrhea, menorrhagia, skin diseases, sore throat</td>
<td>Stem bark</td>
<td>Methanol extract</td>
<td>Acetic acid induced writhing, carrageenan induced paw oedema(^{61})</td>
</tr>
<tr>
<td><em>Carissa carandas,</em> Family: <em>Apocynaceae</em></td>
<td>Used as stomachic, antihelmintic, antiscorbutic and useful in treatment of scabies, pruritus, intestinal worms, sour, fever</td>
<td>Root, fruit</td>
<td>Ethanolic extract</td>
<td>Eddy’s hot plate, carrageenan induced rat paw edema, analgesiometer induced pain, cotton pellet induced granuloma(^{62})</td>
</tr>
<tr>
<td><strong>Cassia sieberiana</strong>, Family: Caesalpiniaceae</td>
<td>Traditional medicine to treat pain and Inflammation</td>
<td>Root</td>
<td>Aqueous extract</td>
<td>Acid induced writhing, carrageenan induced paw edema$^{63}$</td>
</tr>
<tr>
<td>Cussonia paniculata, Family: Araliaceae</td>
<td>Widely used against pain, inflammation, infections</td>
<td>Bark</td>
<td>Aqueous extract</td>
<td>Formalin test, carrageenan and histamine induced edema$^{64}$</td>
</tr>
<tr>
<td>Daphne retusa, Family: Thymelaeaceae</td>
<td>Act as detumescence and acesodyne</td>
<td>Bark,</td>
<td>Ethanol extract and different fractions (pet. Ether, methylene</td>
<td>Carrageenan induced paw edema, ear oedema, acetic acid induced writhing, hot plate test$^{65}$</td>
</tr>
<tr>
<td>Desmodium triflorum, Family: Fabaceae</td>
<td>Used as a remedy for dysmenorrheal, muscle spasms, cough, asthma, diarrhea, dysentery, convulsions, pain</td>
<td>Whole plant</td>
<td>Methanol extract</td>
<td>carrageenan induced paw edema, acetic acid induced writhing, determination of antioxidant enzymes, interleukin-1β, tumor necrosis factor and nitric oxide$^{66}$</td>
</tr>
<tr>
<td>Diospyros variegate, Family: Ebenaceae</td>
<td>Use in relieving fevers and inflammation</td>
<td>Stem</td>
<td>Hexane extract</td>
<td>Acetic acid induced writhing, formalin test, tail flick method, arachidonic acid and ethyl phenylpropionate induced rat ear edema$^{67}$</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Uses</td>
<td>Part</td>
<td>Extract Type</td>
</tr>
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</tr>
<tr>
<td><em>Garcinia hanburyi</em></td>
<td>Family: Guttiferae</td>
<td>Used to treat constipation, edema, bleeding</td>
<td>Gum resin</td>
<td>Ethyl acetate extract</td>
</tr>
<tr>
<td><em>Gloriosa superba</em></td>
<td>Family: Liliaceae</td>
<td>Used in rheumatism, worm infections, leprosy, ulcer, sores, tumor</td>
<td>Aerial parts</td>
<td>Hydroalcoholic extract</td>
</tr>
<tr>
<td><em>Glycine tomentella</em></td>
<td>Family: Leguminosae</td>
<td>Treating degenerative disease, joint pain, joint pain</td>
<td>Root</td>
<td>Aqueous extract</td>
</tr>
<tr>
<td><em>Heracleum persicum</em></td>
<td>Family: Apiaceae</td>
<td>Purposed to reduce swelling, aid digestion and is used as tonic and aphrodisiac</td>
<td>Fruit</td>
<td>Hydroalcoholic extract</td>
</tr>
<tr>
<td><em>Hypericum canariense</em></td>
<td>Family: Clusiaceae</td>
<td>Used in fibromyalgia, arthritis, muscular pain and fatigue, inflammatory and painful conditions</td>
<td>Aerial parts</td>
<td>Infusion, methanol extract and fractions (aqueous, butanol and chloroform fractions)</td>
</tr>
<tr>
<td><em>Hypericum glandulosum</em></td>
<td>Family: Clusiaceae</td>
<td>Used in arthritis, muscular pain and inflammatory and painful conditions</td>
<td>Aerial parts</td>
<td>Infusion, methanol extract and fractions (aqueous, butanol and chloroform fractions)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Use</td>
<td>Plant Part</td>
<td>Extraction Method</td>
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</tr>
<tr>
<td><em>Lactuca sativa</em>, Family: Compositae</td>
<td>Plant seeds are used for relieving pain, osteodynia</td>
<td>Seed</td>
<td>Methanol/petroleum ether (70/30 v/v) extract</td>
<td>Formaline test, carrageenan induced inflammation model&lt;sup&gt;73&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Lactuca scariola</em>, Family: Compositae</td>
<td>Used as a diuretic, antispasmodic, sedative</td>
<td>Seed, stem</td>
<td>Methanol extract</td>
<td>Tail immersion method, carrageenan induced paw oedema</td>
</tr>
<tr>
<td><em>Lantana trifolia</em>, Family: Verbenaceae</td>
<td>Folk medicine use as pain relievers</td>
<td>Leaf</td>
<td>Ethanol extract</td>
<td>Carrageenan, serotonin and histamine induced paw edema, acetic acid induced writhing, tail flick&lt;sup&gt;74&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Leonurus sibiricus</em>, Family: Lamiaceae</td>
<td>Plant is used in the treatment of painful menstruation, post-partum bleeding, oedema</td>
<td>Aerial parts</td>
<td>Methanol extract</td>
<td>Acetic acid induced writhing, carrageenan induced paw edema&lt;sup&gt;75&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Ligularia fischeri</em>, Family: Asteraceae</td>
<td>Seed oil for sprain and rheumatism</td>
<td>Leaf</td>
<td>Ethanol extract</td>
<td>Formalin test, acetic acid induced writhing, hot plate method, carrageenan and arachidonic acid induced edema&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>Species</td>
<td>Usage/Usefulness</td>
<td>Part</td>
<td>Preparation</td>
<td>Test</td>
</tr>
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</tr>
<tr>
<td>Mahonia oiwakensis,</td>
<td>Used as bitter tonic</td>
<td>Root</td>
<td>Ethanol extract</td>
<td>Acetic acid induced writhing, formalin test, (\lambda)-carrageenan-induced paw oedema model$^77$</td>
</tr>
<tr>
<td>Family: Berberidaceae</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Margaritaria discoidea,</td>
<td>Barks are used to relief toothache, post-partum pains, relieve stomach and kidney</td>
<td>Stem bark</td>
<td>Water extract</td>
<td>Carrageenan and histamine induced paw oedema, acetic acid induced writhing, formalin test$^78$</td>
</tr>
<tr>
<td>Family: Euphorbiaceae</td>
<td>disease, inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melia toosendan, Family:</td>
<td>Herbal medicine in the treatment of stomachache and many acute or chronic</td>
<td>Fruit</td>
<td>Ethanol extract</td>
<td>Acetic acid induced vascular permeability and (\lambda)-carrageenan induced hind paw edema, acetic acid induced writhing and hot plate tests$^79$</td>
</tr>
<tr>
<td>Meliaceae</td>
<td>inflammations, as well as ascariasis.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memecylon edule, Family:</td>
<td>In menorrhagia and heavy menstruation, and washing of eyes</td>
<td>Leaf</td>
<td>Hexane, ethyl acetate,</td>
<td>Interleukin production, ethylphenylpropiolate induced ear edema and the writhing test$^80$</td>
</tr>
<tr>
<td>Melastomataceae</td>
<td></td>
<td></td>
<td>methanol and 50% methanol</td>
<td></td>
</tr>
<tr>
<td>Microstylis wallichii,</td>
<td>Useful in haematemesis, fever, vitiated condition of pitta and vata, dipsia,</td>
<td>Tuber</td>
<td>Ethanolic extract (50% v/v)</td>
<td>Carrageenan and cotton palate induced granuloma, pain by analgesiometer$^81$</td>
</tr>
<tr>
<td>Family: Orchidaceae</td>
<td>burning sensation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Uses</td>
<td>Part</td>
<td>Extract Type</td>
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</tr>
<tr>
<td><em>Newbouldia laevis,</em></td>
<td><em>Bignoniaceae</em></td>
<td>Used in earache, sore feet, chest pain, epilepsy, febrifuge, wound and stomach ache</td>
<td>Flower</td>
<td>Ethanolic extract</td>
</tr>
<tr>
<td><em>Pergularia daemia,</em></td>
<td><em>Apocynaceae</em></td>
<td>Used as antihelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent fevers, inflammation</td>
<td>Root</td>
<td>Ethanolic extract</td>
</tr>
<tr>
<td><em>Pfaffia glomerata,</em></td>
<td><em>Amaranthaceae</em></td>
<td>Used in fever and reduce inflammation</td>
<td>Root</td>
<td>Hydroalcoholic extract</td>
</tr>
<tr>
<td><em>Phyllanthus debilis,</em></td>
<td><em>Phyllanthaceae</em></td>
<td>Used in sinusitis, it is a rich source of vitamin c</td>
<td>Whole plant</td>
<td>Petroleum ether extract</td>
</tr>
<tr>
<td><em>Pogostemon cablin,</em></td>
<td><em>Lamiaceae</em></td>
<td>Used in cold, nausea, diarrhea, headache and fever</td>
<td>Aerial parts, leaf</td>
<td>Methanol extract</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Description</td>
<td>Part</td>
<td>Extract Type</td>
<td>Method of Evaluation</td>
</tr>
<tr>
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<tr>
<td><em>Rheedia longifolia,</em> <em>Family: Clusiaceae</em></td>
<td>Different plant from rheedia species used to treat inflammation, pain and infections</td>
<td>Leaf</td>
<td>Aqueous extract</td>
<td>Acetic acid induced writhing, tail flick method, hyperalgesia and pleurisy induced by lipopolysaccharide (^8^6)</td>
</tr>
<tr>
<td><em>Rivea hypocrateriformis,</em> <em>Family: Convolvulaceae</em></td>
<td>Leave juice in rheumatic pain and skin disease of hair scalp</td>
<td>Leaf</td>
<td>Ethanol extract</td>
<td>Tail flick models, carrageenan induced inflammation (^8^7)</td>
</tr>
<tr>
<td><em>Saraca indica,</em> <em>Family: Leguminosae</em></td>
<td>To treat painful conditions, improves digestion and assimilation, alleviates excessive thirst, to kills infectious agents and in blood disease, inflammation.</td>
<td>Leaf</td>
<td>Chloroform, Methanol, water extract</td>
<td>Formalin test, tail immersion method (^8^8)</td>
</tr>
<tr>
<td><em>Smilax china,</em> <em>Family: Liliaceae</em></td>
<td>It is bitter, acrid, anodyne, anti-inflammatory, digestive and used in dyspepsia, flatulence, colic, skin diseases, and fever.</td>
<td>Bark</td>
<td>Aqueous extract</td>
<td>Carrageenan induced paw edema, hot plate method (^8^9)</td>
</tr>
<tr>
<td><em>Spathodea campanulata,</em> <em>Family: Bignoniaceae</em></td>
<td>Plant is uses as astringent and to relief for painful inflammatory conditions</td>
<td>Leaf</td>
<td>Ethanol extract</td>
<td>Acetic acid induced writhing, tail flick method (cold induced), hot plate models, carrageenan induced oedema (^9^0)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Description</td>
<td>Part</td>
<td>Extract Type</td>
<td>Assay Method</td>
</tr>
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</tr>
<tr>
<td><em>Trichilia connaroides</em>, Family: Meliaceae</td>
<td>Used as antihelmintic and used in stomach trouble, wound</td>
<td>Leaf</td>
<td>Chloroform extract</td>
<td>Formaline induced paw edema, acetic acid induced writhing, eddy’s hot plate method&lt;sup&gt;91&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Trigonella foenumgraecum</em>, Family: Leguminosae</td>
<td>Used for stomach upset, swelling, rheumatism, fever and/or lowering blood sugar, and for softening the stool.</td>
<td>Seed</td>
<td>Water soluble partially purified extract (methanol extract subsequently treated with chloroform and acetone)</td>
<td>Acetic acid induced writhing, carrageenan induced edema&lt;sup&gt;92&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Verbena tenuisecta</em>, Family: Verbenaceae</td>
<td>Folk medicine against diarrhea, gastrointestinal disorders, fever, pain, inflammation</td>
<td>Flower bud</td>
<td>Volatile oil isolated by hydrodistillation</td>
<td>Carrageenan induced paw edema, acetic acid induced writhing, hot plate method&lt;sup&gt;93&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Xanthium strumarium</em>, Family: Compositae</td>
<td>Used as anodyne, antirheumatic, appetizer, diaphoretic, diuretic, emollient, laxative and sedative</td>
<td>Fruit</td>
<td>Ethanol extract</td>
<td>Acetic acid induced writhing, croton oil induced ear edema&lt;sup&gt;94&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plant</td>
<td>Use</td>
<td>Extracted Part</td>
<td>Extraction Method</td>
<td>Test Method</td>
</tr>
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</tr>
<tr>
<td><em>Xeromphis spinosa, Family: Rubiaceae</em></td>
<td>Used in pain, inflammation, fever and as aphrodisiac, antiemetic, carminative</td>
<td>Bark</td>
<td>Bark is extracted by ether, ethyl acetate and methanol (1:1:1)</td>
<td>Carrageenan induced paw edema(^{95})</td>
</tr>
<tr>
<td><em>Zizyphus lotus, Family: Rhamnaceae</em></td>
<td>Used in inflammation, stress, tooth pain</td>
<td>Root, bark, leaf</td>
<td>Methanol extract</td>
<td>Carrageenan induced paw edema, tail-flick method(^{96})</td>
</tr>
</tbody>
</table>
1.6 Standardization and Quality Control of Herbal Crude Drugs

Accounting to WHO it is the process involving the physicochemical evaluation of crude drug covering the aspects, as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.

Macro and Microscopic Examination:

For Identification of right variety and search of adulterants.

Foreign Organic Matter:

Remove of matter other than source plant to get the drug in pure form.

Ash Values:

It is criteria to judge the identity and purity of crude drug – Total ash, sulfated ash, water soluble ash and acid insoluble ash etc.

Moisture Content:

To check moisture content helps prevent degradation of product.

Extractive Values:

These are indicating the approximate measure of chemical constituents of crude drug.

Crude Fiber:

To determine excessive woody material Criteria for judging purity.
Qualitative Chemical Evaluation:

It covers identification and characterization of crude drug with respect to phytochemicals Constituent.

Chromatographic Examination:

Include identification of crude drug based on use of major chemical constituent as marker.

Qualitative Chemical Evaluation:

Criteria to estimate amount the major class of constituents.

Toxicological Studies:

Pesticide residue, potentially toxic elements, and Microbial count approach to minimize their effect in final product.

Physical evaluation:

Each monograph contains detailed botanical, macroscopic and microscopic descriptions of the physical characteristics of each plant that can be used to insure both identity and purity. Each description is accompanied by detailed illustrations and photographic images which provide visual documentation of accurately identified material.

Microscopic evaluation

Full and accurate characterization of plant material requires a combination of physical and chemical tests. Microscopic analyses of plants are invaluable for assuring the identity of the material and as an initial screening test for impurities.
Chemical evaluation

A chemical method for evaluation covers the isolation, identification and purification. Chemical analysis of the drug is done to assess the potency of vegetable and animal source material in terms of their active principles. The chemical tests include colour reaction test, these tests help to determine the identity of the drug substance and possible adulteration.

Biological evaluation

Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. All living organism are used, these assays are known as Biological assays or Bioassay.

Analytical Methods

Critical to compliance with any monograph standard is the need for appropriate analytical methods for determining identity, quality, and relative potency. There are a plethora of analytical methods available. However, it is often difficult to know which the most appropriate to use is. The primary goal of AHP is to provide multiple methods of identification and testing by which all aspects of the botanical can be appropriately assayed.

Chromatographic Characterization

Chromatography

Chromatography is the science which is studies the separation of molecules based on differences in their structure and/or composition. In general, chromatography involves moving a preparation of the materials to be separated the "test preparation" over a stationary support. The molecules in the test preparation will have different interactions with the stationary support leading to separation of similar molecules. Test molecules which display tighter
interactions with the support will tend to move more slowly through the support than those molecules with weaker interactions. In this way, different types of molecules can be separated from each other as they move over the support material.

Chromatographic separations can be carried out using a variety of supports, including immobilized silica on glass plates (thin layer chromatography), very sensitive High Performance Thin Layer Chromatography (HPTLC), volatile gases (gas chromatography), paper (paper chromatography), and liquids which may incorporate hydrophilic, insoluble molecules (liquid chromatography).

Purity Determination

Each monograph includes standards of purity and other qualitative assessments which include when appropriate: foreign matter, ash, acid-insoluble ash, moisture content, loss of moisture on drying, and extractives. High performance thin layer chromatography (HPTLC) is valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed sing different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses.

Quantitative Analysis

When applicable, the most appropriate quantitative analytical method with accompanying chromatograms shall be provided. The primary goal of the method(s) is to provide validated methods to be used for the quantization of the compound(s) most correlated with pharmacological activity or qualitative markers as determined by the primary pharmacological literature, constituent declaration in product labeling, and a survey of experts. The method(s) will be
selected from the primary analytical literature by a Methods Selection Committee with priority given to compendial methods when available. In this context, validation consists minimally of a two-lab validation using the same procedures, samples, and reference standards. Primary factors for considering a method as appropriate include accuracy of the findings, speed, basic ruggedness, applicability to a large segment of the manufacturing community and avoidance of the use of toxic reagents and solvents. When necessary, comparative tests shall be conducted to determine which of the available method(s) is most appropriate. The validation process minimally includes: standard precision, linearity, sample precision using replicate samples, sample linearity, selectivity (co-elution, sensitivity to analyte degradation), retention times, and limits of detection. Other methods which may be of value to the industry may be included or cited in the monograph but are not required for compliance with the monograph.

The guidelines set by WHO can be summarized as follows:\textsuperscript{100}:


b. Reference to the physiochemical character of the drug. Chromatographic profiles, ash values, extractive values, refractive index, polarimetric readings, moisture content, volatile oil content, etc.

c. Reference to the pharmacological parameters. Biological activity profiles, bitterness values, haemolytic index, astringency, swelling factor, foaming index, etc.

d. Toxicity details – heavy metals like cadmium, lead, arsenic, mercury, etc. Pesticide residues.
e. Microbial contamination – Total viable aerobic count, pathogenic bacteria like
*Enterobacteria, E.coli, Salmonella, Pseudomonous aeruginosa, Staphylococcus aureus*, etc. and presence of aflatoxins etc