INTRODUCTION

Rheumatoid arthritis has 19th century roots and a 20th century pedigree. Although its name was introduced in the 1850s (Storey GO, et al., 1994), classification criteria were only developed 50 years ago (Ropes MW, et al., 1959 and Arnett FC, et al., 1987). Observational studies in which these criteria are used portray treated rheumatoid arthritis as a serious long-term disease with dominant extra-articular features, limited treatment options, and poor outcomes (Scott DL, et al., 1987 and Pincus T, et al., 1994). Tumour necrosis factor (TNF) inhibitors and other biological agents have heralded a so-called therapeutic revolution, transforming the outlook for patients with rheumatoid arthritis. However, improved disease outcomes preceded biological agents, reflecting early use of conventional drugs, ambitious treatment goals, and better management of co morbidities. An historic parallel is the 1950s revolution in tuberculosis care, when improved conventional management followed by effective chemotherapy made tuberculosis curable (Wilson LG, 2005).

Pathophysiology Rheumatoid arthritis is best considered a clinical syndrome spanning several disease subsets (van der Helm-van Mil AHM, et al., 2008). These different subsets entail several inflammatory cascades (van Oosterhout M, et al., 2008), which all lead towards a final common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present.

When inflammation affects a joint (such as in rheumatoid arthritis), the cartilage can be damaged by neutrophils (Wilson LG, 2005) and lysosomal enzymes (van der Helm-van Mil AHM, et al., 2008) that enter the area. This leads to a vicious cycle of repeated injury and persistent inflammation. Inflammation may also lead to mental depression which is commonly seen in chronic pain patients. Indolamine 2,3 dioxygenase (IDO) is a rate-limiting enzyme in the degradation of tryptophan and is induced during inflammation by the cytokines interferon-gamma (IFN-gamma), interferon-alpha (ITN-alpha) and tumor necrosis factor-alpha (TNF-alpha) in a broad variety of cells. Elevated IDO can therefore enhance tryptophan degradation and subsequent serotonin depletion which may cause depression.
Inflammation one key inflammatory cascade includes overproduction and over expression of TNF (Feldmann M, et al., 1996). This pathway drives both synovial inflammation and joint destruction. TNF overproduction has several causes, including interactions between T and B lymphocytes, synovial-like fibroblasts, and macrophages. This process leads to overproduction of many cytokines such as interleukin 6, which also drives persistent inflammation and joint destruction (Choy EH, et al., 2002). Overproduction of other proinflammatory cytokines (eg, interleukin 1) differs from the process for interleukin 6 in that production is either less marked or is specific to one or more disease subsets, as best shown by the effects of interleukin 1 blockade in sub forms of juvenile idiopathic arthritis or adult-onset Still’s disease.

It is known that excess of reactive oxygen species (ROS) is associated to inflammation, growth and vasoconstriction contributing to vascular injury in many cardiovascular diseases, such as hypertension, hyperlipidemia, and diabetes (Bartsch et al., and Libby, 2006). Inflammation, a self-defense reaction against various pathogenic stimuli, it may become a harmful self-damaging process; if it transforms to chronic inflammation Levine, B et al., 2011). Inflammation is the means by which the body deals with insult and injury. Insult may be caused: mechanically (e.g., by pressure or foreign bodies), chemically (e.g., by toxins, acidity, and alkalinity), physically (e.g., by temperature), by internal processes (e.g., uremia), and by microorganisms (e.g., bacteria, virus, and parasites). Reactive oxygen Species (ROS) and free radicals are thought to act indirectly as cellular messengers and elicit an inflammatory response. Over production of these species may cause oxidative modification of biological molecules eg. trypsin, collagen, LDL, DNA and lipids. ROS and free radicals also activate a series of enzyme systems including protein kinases, protein phosphatases, transcription factors and heat shock proteins. ROS are also critical for gene expression, which encode inflammatory proteins eg. proteinases involved in tissue destruction such as collagenases and gelatinases. Nuclear factor - Kβ dependent. In the case of rheumatoid arthritis, rheumatoid factor binds IgG when it is exposed to free radicals. This binding stimulates the production of more free radicals, which then attack the cartilage matrix (Kumar et al., 2002).
Analgesics reduce pain and non-steroidal anti inflammatory drugs (eg. Aspirin) (NSAIDs) lessen pain and stiffness. Both groups of drugs are used widely to control symptoms of rheumatoid arthritis. Evidence for use of analgesics is modest but uncontroversial (Wienecke T, et al., 2004); support for use of NSAIDs is considerably stronger (Chen YF, et al., 2008). NSAIDs have lost their historical role as first-line treatment because of concerns about their limited effectiveness, inability to modify the long-term course of disease, and gastrointestinal and cardiac toxic effects (Scott PA, et al., 2007 and Schaffer D, et al., 2006). These agents should be given with proton-pump inhibitors for gastro protection, with short-acting drugs administered for short periods to minimize risks.

Disease-modifying antirheumatic drugs (DMARDs) are a heterogeneous collection of agents grouped together by use and convention. They are the mainstay of treatment for rheumatoid arthritis (Donahue KE, et al., 2008). Their diverse mechanisms of action are incompletely understood. They reduce joint swelling and pain, decrease acute-phase markers, limit progressive joint damage, and improve function. Methotrexate is the dominant DMARD. Sulfasalazine and leflunomide are also widely used. Their efficacy has been established in placebo-controlled trials (Donahue KE, et al., 2008, Suarez-Almazor ME, et al., 2000, Osiri M, et al., 2003 and Suarez-Almazor ME, et al., 2000). Hydroxychloroquine and chloroquine have DMARD-like properties. Gold (rINN sodium aurothiomalate) and ciclosporin are additional DMARDs; their use is limited by toxic effects. DMARDs are sometimes combined, and several combinations of DMARDs have proven efficacy (Choy EH, et al., 2005). An example is methotrexate, sulfasalazine, and hydroxychloroquine - termed triple therapy. Use of DMARD combinations varies across different countries; in some regions they are used rarely. Adverse effects of DMARDs include those that are minor (eg, nausea) and serious (eg, hepatotoxicity, blood dyscrasias, and interstitial lung disease) (Salliot C, et al., 2009 and Alcorn N, et al., 2009). Monitoring of adverse effects requires pretreatment screening and subsequent safety recording of blood counts and liver function tests (Chakravarty K, et al., 2008). Because of the side effect profile of NSAIDs and DMARDs, patients are inclined to choose the alternative system of treatment.
The Ayurvedic concept appeared and developed between 2500 and 500 BC in India. The literal meaning of Ayurveda is “science of life,” because ancient Indian system of health care focused views of man and his illness. It is pointed out that the positive health means metabolically well-balanced human beings. According to Ayurveda, the disease evolves from the body due to external factors. It has a vast literature in Sanskrit covering all aspect of diseases, pharmacy and therapeutics. The practice of Ayurveda therapeutics consisted of 8 sections divided into 180 chapters and listed 314 plants, which are used as medicines in India (Subhose, et al., 2005). The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Ballabh, and Chaurasia, 2007). Many Westerners have long regarded the Indian systems of medicine as a rich source of knowledge (Subhose, et al., 2005).

Even today, majorities of the medicines are prepared from the plant and animal products, minerals and metals etc. Major pharmaceutical industries depend on the plant products for the preparation of Ayurvedic medicines. In the present context, the Ayurvedic system of medicine is widely accepted and practiced not only in the Indian Peninsula but also in the developed countries such as Europe, United States and Japan. Plant derived medicines have been the first line of defense in maintaining health and combating diseases (John, 1984 and Veale, et al., 1992). In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from the traditional healers (Anesini, and Perez, 1993). Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants (Cox, 1990 and Cox, 1994).

It is unambiguous that the survival and enhanced life span of human beings is made possible by the medicinal plants. The dependence of early man on medicinal plants is as old as the civilization itself. The therapeutic property of the medicinal plants is the outcome of the active constituents; these pharmacologically active constituents were synthesized and stored in different plant parts. Researchers are trying to explore this treasure of bio active molecules to convert the natural chemicals in a form useful for modern systems of medicine. The chemical constituents of herbal drugs were believed to have better compatibility with the human body and hence less
side effects associated with them. Hence there is a growing trend in screening new
herbs with subsequent isolation of the bioactive molecules from them. The traditional
medicines have been derived from rich traditions of ancient civilizations and heritage.
Indigenous systems of medicine across the world have enriched the present
knowledge about the secondary metabolites and hence much of the scientific
investigations are associated and relay on traditional systems of medicine. Indian
system of medicine is considered as one of the richest ethnobotanical source and the
work presented herein is a study carried out on a plant frequently used in Ayurvedic
system of medicine.

Biologically active compounds from natural sources have always been of great
interest to scientists working on infectious diseases. Research to find out scientific
evidence for claims of plants used for Indian Ayurvedic system of medicine has been
intensified. Detailed research on the chemistry and pharmacology of products of plant
origin are much essential and this may eventually lead to the discovery of medicine
that can be used in the treatment of several diseases (Dev, 1997). Moreover, these
local Ayurvedic preparations are scientifically evaluated and disseminated properly,
our indigenous population can be given better access to efficacious drug treatment and
improved health status (Manandhar et al., 1985 and 1987). However, over
commercial exploitation of these plant (herbal) products frequently degradation of
natural resources are reported to be major threats to medicinal plants in India.

India’s State Governments administer a modern medical infrastructure for
health delivery services in rural areas including district hospitals, block-level
hospitals, primary health centres, primary health units (for every 5,000 people), and
village health guides (for every 1,000 people). However, it is believed that a
maximum of less than half of the rural population is actually covered by the health
services - in certain areas the coverage is very low. Due to the shortcomings of the
modern health system, a number of World Health Organisation (WHO) resolutions
(WHO 29.72, 32.42, 30.49, & 31.33) emphasise the need for wider use of traditional
medicine, urging the member countries to promote traditional medicinal systems.
Hereby attention is focused on the importance of medicinal plants in health care
systems of the developing world. In this context, indigenous Indian health systems,
which are based on local resources such as medicinal plants and have cultural roots in
the communities, could be a valuable supplement to the modern health care system.
The indigenous health systems in India have two social streams: One is folk medicine or Local Health Traditions (LHT), which are oral traditions found in the rural communities all across India. The carriers are millions of housewives with practical knowledge of simple home remedies, traditional birth attendants, local healers, bone-setters, practitioners skilled in acu-pressure, eye diseases, dental care, poisons or veterinary care and village-level herbal medicine healers. They constitute an autonomous, self-reliant and community-supported system of health delivery at the village level. The folk medicine is based on empirical knowledge and utilises a large proportion (25-60%) of local plant species in the various regions, as well as many animals and some minerals.

The second stream of the indigenous health system consists of traditional, organised and codified Indian Systems of Medicine (ISM) such as Ayurveda, Unani, Siddha and Amchi. Unlike the folk medicine, these systems have sophisticated theoretical foundations expressed in hundreds of manuscripts covering treatises on all branches of medicine and surgery. Some of the Ayurvedic texts are up to 3,000 years old. Herbal medicine is dispensed regularly and the recipes are usually quite complicated, comprising 20-30 different plant species each. The total number of plant species used in ISM is much lower than in folk medicine; in Ayurvedic texts, approximately 600 species are mentioned.

The primary users and producers of medicinal plants are the tribal and nontribal rural poor, who are outside the official primary health care system. Both of these groups possess much knowledge about traditional medicine. The people, living in the areas with a high density of medicinal plants, are mainly tribal people. Mostly, elders and housewives possess the knowledge of medicinal plants. However, due to the past century of exploitation of tribals by outsiders, they often refrain from sharing their knowledge with others. They are afraid of witnessing even further exploitation. Each tribal group has its specialization on medicinal plants and the knowledge is mostly kept exclusively within the group. The impact of modern medicine is considerable and the usage of herbal medicine is steadily eroding. The status of local health traditions is often low in relation to modern medicine. People who can afford modern medicine and most young people are more attracted to urban (western) lifestyles and values.
The elders have often given up trying to educate them about the uses of medicinal plants because of their lack of commitment and willingness to enter the long-term process of learning. If this problem is not addressed, the medicinal knowledge may die out due to the fact that the elders do not feel secure about sharing this valuable knowledge with others. The elders are afraid, that their learnings could be improperly handled or even misused. Access to the official medical centres in rural areas, where western medicine is available, is often difficult for the rural poor. Although medicine and treatment is relatively cheap in absolute terms, it is often beyond the reach of poor people. In the opinion of some social workers, loans to cover medicinal expenses are considered to be a major debt burden for many people in the rural areas. The traditional health sector in India may thus play a substantial role in providing health care to the Indian People, and has the potential of further contributing to the self-reliance of village communities, if a continuous effort is made to revitalise its folk stream.

The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17,000 species of higher plants, of which approximately 8,000 species are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems, such as the Ayurveda. The medicinal plants are listed in various indigenous systems such as Siddha (600), Ayurveda (700) and Amchi (600), Unani (700), Allopathy which 30 plant species for ailments (Rabe and J. V. Staden, 1997). Many of the wild plants are endemic and are found only in specific ecological niches. Due to the 250 per cent increase in human and livestock populations in the 20th century and the subsequent pressure on available land, which has lead to deforestation and land degradation, many species or populations of species are now threatened with extinction, because their natural habitats are being destroyed. Almost all medicinal plant raw materials in India are collected from wild populations. This has led to the unsustainable exploitation of many of the plants. The growing interest in traditional herbal medicine will lead to a further increase in the demand for medicinal plants. The aim of the present study is to understand the knowledge of plant Delonix elata that have been used against inflammation by local people.
*Delonix elata* (Caesalpiniaceae) is a small sized tree found in Gujarat, Western peninsular and Southern India. Trunk of the tree is smooth, ash coloured, leaves compound, rachis 15-30 cm long, bipinnate, leaflets 10-20 pairs, flowers yellowish white in terminal corymbiform racemes, pods small, 12-18 cm long, seeds 4-8 (Cooke, 1903 and Shah, 1978). *D. elata* is native to Madagaskar, later introduced and naturalized in India, commonly known as “Sandesaro”. It is not a classical Ayurvedic drug but found included in Shodhala Nighantu under the Sanskrit name of “Siddeshwara” during 12th century AD (Sharma, 1978). The leaves of which are used both internally and for external application in cases of inflammatory joints by applying paste or by taking the expressed juice by local people. Medicated oil prepared from the leaves is marketed under the name of “Vathanarayana”. Leaves are used as a folklore remedy for inflammatory joint disorders (Samvatsar, Diwanji, 1999). *Delonix elata* (*D.elata*), is one of the plants that has long been used in traditional herbal medicine for the treatment of arthritic pain.

The leaves (Kirtikar, and Basu, 1984 and Anon, 1952) are said to be used in the Indian indigenous system of medicine for rheumatism and in fevers. The bark is considered febrifuge and antiperiodic. Earlier report (Subramanian, and Ramakrishnan, 1968) on bark of this plant shows the presence of amino acids. The aqueous extract of leaves (Sethuraman, and Sulochana, 1986) of this plant produced a dose-dependent inhibition of carrageenin-induced rat foot oedema. The activity has been attributed to the presence of flavonoids. The flowers of *Delonix elata* *D.elata* collected from Pondicherry were reported to contain quercetin 3-0-glucoside by Subramanian and swamy (Subramanian, and Narayanaswamy,. 1963). The leaves of this plant collected from Trichy have been found to contain quercetin 3-0-galactoside and rutin (Sethuraman, and Sulochana, 1986). In order to ascertain any geographic variation in the flavonoids constituents of *Delonix elata* *D.elata* and to find the presence of any other phenolics, it was considered desirable to examine the ivory white flowers of this species, available in Pondicherry. (E)-catfeic and 2',4,4', 6'-tetrahydroxychalcone 2'-glucoside (isosalipurposide) along with a flavonol -3,5,7,3',4'-pentahydraxyflavone (quercetin) – and two of its glycosides-quercertin 3-0-rutinoside (rutin) and quercetin 3-0-galactosi.de (hyperoside) - were isolated from the ivory white flowers of *Delonix elata*. 
Delonix elata is an endangered deciduous tree is under immediate threat as a source of timber and overgrazing is another causative for its decline (Hadidi, et al., 1991). D. elata is a multipurpose tree. It is a promising source of micronutrients for goats, sheep, camels and cattle, which eat the foliage and young pods. It is very promising as a firewood source. The tree yields a dark coloured, mucilaginous gum. D. elata has a potential use in soil erosion control. Also, it is a good tree for reforestation of difficult sites. The medical usefulness of the tree is acknowledged, the decoction of the leaves are used to get relief from rheumatic problems like pain and stiffness of the joints, especially the knees. The root decoction is drunk for abdominal pains. Seed oil of D. elata contains small amounts of sterculic and malvalic acids (Daulatabad, et al., 1987). D. elata leaves contains high levels of phenolic compounds and mould (Shayo, and Uden, 1999). Also, Goromela et al., indicated that irrespective high concentration of phenolic compounds-amyrin, hesperitin and neohesperidin were isolated from the dried roots of D. elata.

Delonix elata also commonly known as Perungondrai tree (in Tamil) most commonly used household remedy for many manifestations. Delonix elata is known to be used for joint pains and in flatulence. The medical usefulness of the tree is acknowledged by people living in the villages who take a decoction of the leaves to get relief from rheumatic problems like pain and stiffness of the joints, especially the knees (Thirugnanam, et al., 2003). It has been used in the treatment of bronchitis, stomach disorders (Thirugnanam, et al., 2003) and pneumonia in infants. Leaf extracts of D. elata are reported for strong anti-inflammatory activity (Sethuraman, et al., 1986).

Phytochemicals, especially flavonoids and phenolic acids, are of current interest because of their important biological and pharmacological properties. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). This is in contrast to primary products, such as carbohydrates, lipids, proteins, heme, chlorophyll, and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman et al., 1999; Wink, 1999). Although plant secondary products have
historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the ecophysiology of plants. Accordingly, secondary products have both a defensive role against herbivory, pathogen attack, and inter-plant competition and an attractant role toward beneficial organisms such as pollinators or symbionts (Kaufman et al., 1999; Wink and Schimmer, 1999). Plant secondary products also have protective actions in relation to abiotic stresses such as those associated with changes in temperature, water status, light levels, UV exposure, and mineral nutrients (Kaufman et al., 1999). Furthermore, recent work has indicated potential roles of secondary products at the cellular level as plant growth regulators, modulators of gene expression, and in signal transduction (Kaufman et al., 1999).

Inflammation is a rapid response of tissue to injury and characterized in the acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leucocytes, and inflammatory mediators, such as cytokines such as TNF-α, IL-6, IL-1β (Feghali, C.A and Wright, 1997). The excessive production of these cytokines may result in the systemic inflammatory response syndrome (SIRS), severe tissue damage, and septic shock (Shimazu, R et al., 1997 and Beutler, B et al., 1986). Among the cytokines, TNF-α is thought to be one of the most important mediators of inflammatory diseases. It is elevated in some pathogenic conditions and possesses potential toxic effect that results in hypersensitivity reactions with chronic inflammation (Morrison, D.C et al., and Levine, B et al., 1990). IL-6 is a cytokine produced by a number of normal and transformed cells. It is believed to be an endogenous mediator of LPS-induced fever (Bartold, P.M et al., 1991). IL-1 is a multifunctional cytokine that is responsible for various processes including host defense, inflammation and response to injury. It is produced by many cell types, predominantly by macrophages (Kielian, T et al., 2004). In recent years, people began to use extracts from natural medicinal plants to prevent and treat inflammatory responses by inhibiting inflammatory cytokines, such as TNF-α, IL-1β and IL-6, and this has become an important area of investigation.

The application of computational methods to study the formation of intermolecular complexes has been the subject of intensive research during the last decade. It is widely accepted that drug activity is obtained through the molecular binding of one molecule (the ligand) to the pocket of another, usually larger, molecule
(the receptor), which is commonly a protein. In their binding conformations, the molecules exhibit geometric and chemical complementarity, both of which are essential for successful drug activity. The computational process of searching for a ligand that is able to fit both geometrically and energetically the binding site of a protein is called molecular docking.

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

One key aspect of molecular modelling is calculating the energy of conformations and interactions. This energy can be calculated with a wide range of methods ranging from quantum mechanics to purely empirical energy functions. The accuracy of these functions is usually proportional to its computational expense and choosing the correct energy calculation method is highly dependent on the application. Computation times for different methods can range from a few milliseconds on a workstation to several days on a massively parallel supercomputer. In the context of docking, energy evaluations are usually carried out with the help of a scoring function and developing these is a major challenge facing structure based drug design (Vieth et al., 1998). Scoring functions are a critical part of the structure based drug design process. No matter how efficient and accurate the geometric modeling of the binding process is, without good scoring functions it is impossible to obtain correct solutions. The two main characteristics of a good scoring function are selectivity and efficiency. Selectivity enables the function to distinguish between correctly and incorrectly docked Structures and efficiency enables the docking program to run in a reasonable amount of time.
Plate 1: Selected medicinal plant - *Delonix elata*
Aim and Objectives

The present study is aimed with following objectives to assess the medicinal properties hidden in Delonix elata in order to validate its therapeutic effect against collagen induced rheumatoid arthritis.

1. Screening of phytochemical and biochemical constituents present in Delonix elata leaves.
   a. Screening of secondary metabolites in Delonix elata leaves.
   b. Phytochemical characterization of Delonix elata methanolic leaf extract by Gas Chromatography - Mass Spectrometry (GC-MS) studies.


3. To assess the acute anti-inflammatory effect of leaf extract of Delonix elata in collagen induced arthritic mice.

4. Chronic in-vivo study and animal experimentation of leaf extract of Delonix elata in Collagen induced arthritic mice.

5. Molecular docking study carried out for the identification of docking ability of lead compounds in Delonix elata.