Chapter - VI

Summary
Herbal medicines have been used world-wide for thousands of years. These herbs mainly originate from plants, minerals and animal products, and may be used either in their primary forms or combined into mixtures. Herbal preparations can also be formulated into tablets, pills, and liquids, as well as being commercially available in the form of proprietary medicines. Traditionally, herbs have been considered to be gentle, non-toxic and even harmless, mainly because of their natural origin (Jou-Fang, 2002). The widely recognized international concern is the crisis of new infectious diseases which no effective chemo-therapies are available. The new drugs from herbal medicines can be one of the alternatives for this difficult problem; indeed, all herbal medicines represent a virtually untapped reservoir of new drugs (Don et al., 2002). The evaluation of efficacy and safety of these herbal remedies is now being approached with the methodologies and clinical trial protocols standard in Western medicine (Xiu-Min, 2007).

Quality control for herbal preparations or proprietary products, however, is much more difficult than for synthetic drugs because of the chemical complexity of the ingredients. As herbal preparations comprise hundreds of mostly unique, or species-specific, compounds, it is difficult to completely characterize all of these compounds. It is also equally difficult to know precisely which one is responsible for the herbs or herbal preparation's therapeutic action because these compounds often work synergistically in delivering therapeutic effects. Thus, maintaining consistent quality in herbal preparations, both from batch to batch and over time, is as problematical as it is necessary and has drawn serious attention recently as a challenging analytical task (Ying et al., 2007). Determining the effective or principal chemical constituents and the toxic compounds is crucial to the quality control of herbs and herbal preparations (Li et al., 1998). It is now been universally accepted fact that the plant medicines and remedies are far safer than that of synthetic
drugs for curing the serious diseases like cancer, hepatitis, arthritis, epilepsy, etc. The plants are currently being used in the treatment of various disease conditions without standardization.

One such medicinal plant is *Kigelia reticulata*, which is having various ethanobotanical importances in Western Ghat region. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The result of our pharmacognostic investigations could therefore, serve as a basis for proper identification, collection and investigations of the plants in the future days. The macro and micromorphological features of the leaf described, the fluorescence behaviour, qualitative leaf microscopy and physicochemical studies are parameters that are unique to the plant and are required in its standardization. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. Presence of various secondary metabolites, make the plant useful for treating different ailments and having a potential of providing useful drugs of human use. The plant was further subjected to phytochemical analysis and various pharmacological activities such as analgesic, anti-inflammatory, anti-arthritis, antimicrobial, antioxidant and anti-helminthic screening.

With the results obtained from analgesic activity, it is evident that ellagic acid and methanol extract can produce appreciable effects compared to other compounds and chloroform extract while using both acetic acid-induced writhing and formalin test in rats. Ellagic acid was able to block both phases of the formalin response but the effect was more prominent in the second phase. In the present study, ellagic acid showed maximum protection against formalin induced writhing followed by other models probably explained the peripheral analgesic potential of its prostaglandin inhibitory activity.
Our present study establishes the anti-inflammatory activity of the ellagic acid and methanol extract in the model used. The results of Carrageenan induced edema prove that ellagic acid and methanol extract to be potent anti-inflammatory agents. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito and Antonio, 1998). These results indicate that the test drugs acts in later phases probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization (Just et al., 1998).

Herbal remedies have also been reported to be effective in controlling inflammation for acute soft tissue injuries. Various herbal prescriptions have been found effective in reducing swelling, pain and improving joint mobility in acute joint sprains (Lam, 2001; Ko and Wong, 2002). Our study addresses the basic scientific effects of herbal drug and will pave the way for further clinical studies and evaluations to justify the use of plant as an anti-arthritis agent. From the above studies on arthritic model it can be proposed that ellagic acid can be a possible novel drug for arthritis and also methanol extract along with its various constituents encourage it to be an arthritic agent; and the same is correlated with in silico analysis.

Changes in body weight have also been used to assess the course of the disease and the response, as it is dependent on the alterations in the metabolic activities of diseased rats (Eric and Lawrence, 1996). There was an increase in the body weight of ellagic acid treated animals which is attributed to the reason that it may be due to the restoration of the absorption capacity of the intestine. Joint protection plus suppression of synovitis are known to be the ultimate goals (Atzeni and Sarzi, 2007) of a better osteo arthritic treatment and the ellagic acid achieved these goals.
In the articular chondrocytes in the synovial joint HIF-2α promotes degradative pathways fostering osteoarthritis. Therefore, a disease progression model was developed to provide an understanding of the relationship between target modulation and efficacy in the animal model. From the in vitro anti-arthritis results obtained, our study reveals that ellagic acid is capable of controlling the production of auto antigen and inhibits denaturation of protein, membrane lysis and proteinase action in rheumatic disease. Obtained data correlates with in vivo and in silico activity conducted and states that ellagic acid can be used as potent anti-arthritis agent.

Antibacterial activities were performed against both Gram positive and Gram negative organisms. The tested compound polyprenol showed varying degree of antibacterial activities against the bacterial species. A correlation was found between the antibacterial activity observed by agar diffusion assay and MIC, MBC determination which was the same case observed with Ramzi et al., (2009). With the antibacterial study conducted, polyprenol showed to have very strong inhibitory capacity which is followed by methanol extract; and is again supported by automated docking process. The docking of polyprenol with Glutamine amido transferase domain reveals that, our compound exhibited interactions with one or the other amino acids in the active pocket. By in silico analysis, it seems that polyprenol is promoting the remarkable antibacterial activity through the inhibition of GlcN-6-P synthase. The results of our study also revealed that polyprenol and methanol extract showed good antibacterial activity against Methicillin-Resistant Staphylococcus Aureus and good inhibition of biofilm formation by clinical isolates of MRSA. Hence, polyprenol has been proved to be one of the potent antibacterial agents.

The different solvent extracts of K. reticulata leaves were screened for their in vitro antioxidant activities. Extracts showed promising results for total antioxidant capacity and reductive capability comparably. The
ethanol extract was found to possess excellent antioxidant activities. It was also focused to determine the total phenolic and flavonoid content present, which showed promising results in the ethanol extract. The antioxidant property may be attributed to the presence of flavonoids and phenolics present in the drug. The ability of the crude extracts towards reduction, presence of phenol, flavonoid and antioxidant is an indication of its broad spectrum potential which may be employed in the management of various diseases. Our study proves that the plant contains good store of antioxidants and other essential metabolites to support its efficiency to be a drug.

The antihelminthic activity carried out with polyprenol at various concentrations, against the worm *Pheretima posthuma* showed very promising results. The results of present study indicated that the polyprenol significantly demonstrated paralysis and also caused death of worm in dose dependent manner. In the molecular docking studies of polyprenol with the catalytic site of tubulin, the best docked conformation of ligand–receptor complex was determined based on the lowest interaction energy and binding free energy. This work also helps in understanding the potential development of parasite-specific target based drug. Polyprenol showed significant antihelminthic activity *in vitro* and *in silico*; hence, it can be used as an effective drug against parasitic infections of humans.