



**"CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN HERBAL REMEDIES  
FOR ARTHRITIS MANAGEMENT: A COMPREHENSIVE ANALYSIS USING  
ADVANCED CHROMATOGRAPHIC TECHNIQUES"**

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**ABSTRACT**

Arthritis is a prevalent chronic inflammatory disorder affecting millions worldwide, leading to significant morbidity and decreased quality of life. While conventional treatments are available, their long-term use often comes with adverse effects. As a result, traditional herbal remedies have gained increasing attention as alternative or complementary therapeutic options for arthritis management. This study aims to isolate and characterize the active compounds present in a selected herbal medicine renowned for its efficacy in alleviating arthritis symptoms. In this research, various traditional medicinal plants with reputed anti-arthritic properties were collected, and their extracts were prepared using suitable solvents. The herbal formulation under investigation was derived from a combination of these plant extracts, enhancing the potential for synergistic effects. The process of isolation involved advanced chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) to identify and separate individual bioactive compounds.

**Keywords:** - Herbal, Chronic, Isolation, Medicines, Diseases.

**I. INTRODUCTION**

Herbal medicines play a major role in health-care programmes in developing countries. Around 80% of world population still depend on medicinal plants for their primary health-care needs. India is recognized as a land of herbal plants; hence, any specific data on such plants could be of clinical importance. Eco-friendly and bio-friendly plant based commodities has recently been given consideration for the prevention and treatment of various infections including microbial diseases throughout the world and employment of plants in ethno medicine is on the rise worldwide.

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Medicinal plants are a

natural gift for human being's disease free and healthy life. The herbal products today are considered to be safer to human and environment. In India different parts of several medicinal plants or their extracts are used for the treatment of various diseases. The extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity drug profile.

Over the years, medicinal plants have been found useful in the treatment and management of various health problems. In recent years, there has been an increasing interest by researchers in the use of naturally occurring biologically active compounds of medicinal value.[4] Before the discovery of synthetic drugs man was completely dependent on the



medicinal plants for the treatment of diseases. The medicinal value of plants has been recognized by every person of this society.

In the nineteenth or earlier centuries natural product extracts, particularly those derived from botanical species, provided the main source of folk medicines. However, in the later part of the nineteenth century, biologically-active organic molecules began to be isolated in relatively pure form for medicinal use. For example, salicylic acid, the precursor of aspirin, was isolated in 1874 from Willow bark.

## **II. MONONUCLEAR PHAGOCYTE SYSTEM (MPS)**

### **Role of Monocytes in Chronic Inflammation**

Among the cells in inflammation, mononuclear phagocyte system (MPS) plays a pivotal role in the development, scavenging, inflammation, and phagocytosis and in organizing various phases of inflammation. MPS comprises group lineage committed bone marrow precursors, monocytes, resident macrophages and dendritic cells (Italiani & Boraschi, 2014). Monocytes constitute 10% of the circulating peripheral blood leukocyte originating from bone marrow from among common myeloid progenitor cells. Circulating monocytes are nondividing cells with a half-life of 3 days.

### **Monocyte-derived Macrophages (MDMs)**

There is always the presence of tissue-resident macrophages maintained by local proliferation and is involved in the tissue homeostasis and resolution of inflammation and are known by different names depending on the tissue/organ where they are residing. But during

inflammatory conditions, the inflammatory stimuli results in the rapid recruitment of monocytes into the site of inflammation.

### **Monocyte-derived dendritic cells (MoDCs)**

Dendritic cells are specialized immunological cells involved in the induction and regulation of immune response (Kalinski, Muthuswamy, & Urban, 2013). At the site of inflammation, the exposure of monocytes to GM-CSF and IL-4 results in differentiation of monocytes to monocytes derived dendritic cells (MoDCs). The inflammatory DCs so derived have phenotype and functions of DCs like the ability to process and present antigen to T cells.

### **LPS stimulation of monocytes/ MDMs, MoDCs**

LPS stimulated MPS is one of the widely used models to study the anti-inflammatory potential of compounds invitro. LPS initiates an inflammatory response in monocytes via its interaction with TLR4 complex. This results in the activation of downstream processes involving MAPK pathways through an IRAK-1 mediated pathway which leads to the synthesis and secretion of various pro-inflammatory and anti-inflammatory mediators including cytokines.

## **III. PROINFLAMMATORY ENZYMES LIPOXYGENASE**

Leukotrienes (LTs) are a group of lipid mediators which plays a pivotal role in the pathogenesis of chronic inflammatory diseases like rheumatoid arthritis, asthma, inflammatory bowel disease (J. N. Sharma & Mohammed, 2006; Whitehouse & Rainsford, 2006), atherosclerosis, dermatitis, CVD and cancer (Wisastra & Dekker, 2014). Leukotrienes are synthesized by the lipoxygenase from



arachidonic acid released from the cell membrane by the action of PLA2. Lipoxygenase is a family of non hemecontaining deoxygenase that can act on PUFA containing at least two isolated cis-double bonds to form various hydroperoxy derivatives. Besides arachidonic acid, mammalian LOX can act on linoleic acid as a substrate. The products of LOX are various lipid mediators having a role in inflammatory processes like leukotrienes, lipoxins, hepoxilins, eoxins, resolvins, and protectins. Beside the mentioned classical role of LOX in metabolism of AA, increased LOX activity can lead to cellular dysfunction by oxygenating polyenoic fatty acid thereby leading to formation of hydrophilic pores in the cell membrane. Further, the hydroperoxy lipids formed by LOX can alter the gene expression of redox-sensitive genes. In humans, six functional genes (ALOX15, ALOX15B, ALOX12, ALOX12B, ALOXE3, ALOX5) found in chromosome 17 (except ALOX 5 which is situated in chromosome 10) codes for six different LOX. ALOX 15 codes for 12/15LOX which is highly expressed in eosinophils, bronchoalveolar epithelial cells and in IL-4 treated monocytes, ALOX15B codes for 15-LOX2 expressed in epithelial cells, ALOX12 codes for platelet 12 LOX in blood platelets and in skin, ALOX12 B codes for 12R lipoxygenating enzyme, ALOXE3 gene encodes two distinct epidermis type LOX isoforms in skin and the most important ALOX5 gene in leukocytes, monocytes, macrophages and dendritic cells codes for 5-LOX which plays major role in leukotriene synthesis (Kuhn, Banthiya, & van Leyen, 2015). LTs also activates the proinflammatory signaling pathway NF-

κB (Wisastra & Dekker, 2014) Due to the important role of leukotrienes, developing LTs modifiers, LT inhibitors or antagonist is considered an important therapeutic approach in the management of chronic inflammation (J. N. Sharma & Mohammed, 2006). Some of the currently marketed lipoxygenase inhibitors are – antimetabolites (eicosatetraynoic acid, ETYA), Anti-inflammatory/ analgesics drugs (eg: benoxaprofen, diflunisal, sulphasalazine), 5 LOX inhibitors like zileuton, L-739,010, MK886 and natural products from traditional medicines like boswellic acid, NDGA, dromaiol, lyprinol, curcumin, Withania.

#### IV. CYTOKINES IN CHRONIC INFLAMMATION

##### Interleukin-1β

IL-1β is one of the key cytokine involved in the pathogenesis of chronic inflammatory conditions synthesized by monocytes, macrophages, dendritic cells, chondrocytes, osteoblast and cells of synovial membranes. IL-1β is formed as a cytosolic precursor pro-IL-1β (269 aminoacids) which are converted to active IL-1β (153 aminoacids) and released into extracellular space by the action of proteolytic enzyme Caspase 1(IL-1β converting enzyme).

##### Tumor Necrosis Factor-α

TNF-α is a potent mediator of inflammation along with IL-1β and are important in maintaining homeostasis and in pathogenic immunity. TNF-α is generally expressed by activated monocytes, macrophages, NK cells, T cells, endothelial cells and by fibroblast. It is expressed as pro-TNF, a transmembrane protein (26KDa) which is converted to soluble TNF (17KDa) by the action of metallo protease TACE (TNF-α converting



enzyme). The downstream signaling is carried out by its interaction with the TNFR1 and TNFR2 receptors which results in the activation of NF- $\kappa$ B and several additional pathways.

### V. AYURVEDA

WHO defines traditional medicines as diverse health practices, approaches, knowledge and beliefs incorporating plants, animals, and or mineral based medicines, spiritual therapies, manual techniques and exercise applied singularly or in combination to maintain well being, as well as to treat, diagnose or prevent illness (Patwardhan, B.Vaidya, Chorghade, & Joshi, 2008). Ayurveda is the traditional system of medicine prevalent in India, Srilanka and other South Asian countries having a history which dates back to 5000 B.C (Mukherjee & Wahile, 2006; Patwardhan, Vaidya, & Chorghade, 2004). Around 75-80% of rural population in India still relies on ayurveda for treatment (Garodia, Ichikawa, Malani, Sethi, & Aggarwal, 2007). According to Astangahridayam, the classical ayurvedic textbook, Ayurvedic mode of treatment believes in cause-effect phenomenon and accordingly the therapies are classified as 'Yukti vyapasraya' (rational therapies), satvavajaya (psychological therapies) and daivyavyapasraya (divine methods like faith healing). Ayurveda states the body is governed by three principles called as 'tridosha' (Kapha, pitta and vata) and any disease (roga) occurs due to an imbalance of tridosha. Based on the tridosha an individual or body can be classified into Vata, Pitta, Kapha prakruthi tridosha body type or can be dual type (Vata-pitta, pitta-kapha or vata-kapha). Among this tridosha Kapha prakruthi are considered good while Vata prakruthi and dual types are

considered to be prone to diseases. Ayurveda principle believes that the imbalance in the prakruthi is caused by external factors (agantu roga) like injuries and by internal factors (nija roga) as excessive eating. The treatment regime in Ayurveda is individually customized based on the prakruthi of the person. The ayurvedic treatment aims to bring the tridosha to normal levels. It may be by 'shamana'. i.e., palliative treatment in initial stages which aims to bring the doshas to normalcy and 'shodhana' which involves purification treatment which expels imbalanced doshas out of the body in chronic stages of the disease. For example, for vata dosha, during shodhana, basti (suppositories) is carried out and oil as shamana. For pitta, virechana (purgation) and ghee are used as shodhana and shaman respectively and for kapha, vamana (emesis) is shodhana and honey is shamana (Vagbhat, n.d.).

### VI. CONCLUSION

Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as they have fewer adverse effects as compared with modern medicines. The drugs of Ayurvedic origin can be utilized in a better form with enhanced efficacy by incorporating in modern dosage forms. Phytotherapeutics deliver the components in a novel manner to increase patient compliance and avoid repeated administration. More plants and their combinations should be explored to get reliable, safe and effective formulations that can compete with synthetic drugs.

In the present study, the medicinal plants *Cardiospermum halicacabum* and *Drynaria*



quercifolia that are well established for the treatment of arthritis were selected and formulated as transdermal patches.

Prior to preformulation and formulation development, the selected powdered plant drugs were subjected to Phytochemical screening after successive solvent extraction. Qualitative chemical examination of extracts revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins. Based on phytochemical studies, alcoholic extract was selected and subjected to formulation development.

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