PREFACE

In the recent years the requirement of anti-inflammatory medication is still the mainstay of most classically taught clinicians for joint and spine related inflammatory pain, despite of their commonly known side effects. There is a huge requirement of herbal and natural products are having anti-inflammatory and anti-oxidant properties without any adverse effects. *Annona squamosa, Annona reticulata* and *Annona muricata* are important edible and medicinal plants in Indian traditional system of medicine. The leaves of these plants are used by the tribal community in India for anti-inflammatory, anti-lice and mosquito repellent properties.

The review of literature, phytochemical analysis, *in vitro* screening for different activities, toxicity and mutagenicity properties, antipyretic, acute and chronic inflammatory models by treatment of plant extracts and their possible outcomes. Inflammation is typically a body’s self-protective mechanism that is triggered in response to noxious stimuli or infection to guard the body and to accelerate the recovery process. However, the neglected acute inflammation leads to the chronic inflammation disorders and arachidonic acid (AA) metabolism plays a crucial role in inflammatory process and associated diseases. Most of the anti-inflammatory medications inhibit the lipoxygenase or cyclooxygenase pathways and these two pathways are potential interventions against inflammation. Most of the anti-inflammatory drugs such as steroids and COX inhibitors are often associated with adverse effects like gastro intestinal irritation, ulcers, hypertension and cardiovascular problems.

Moreover, natural products are widely recognized in the pharmaceutical industries for their broad structural diversity as well as their wide range of pharmacological
activities. With the development of molecular biological techniques and the advances in genomics, the majority of drugs discovery programmes are currently based on the molecular approaches. The molecular mechanism involved in anti-inflammatory response by phytomedicine shares common molecular targets with non-steroidal anti-inflammatory drugs, as well as steroidal drugs. Both non-steroidal anti-inflammatory drugs and phytomedicine constituents are able to inhibit process that leads to inflammation, such as:

- Up-regulation of arachidonic acid metabolites by increasing its metabolism
- Up-regulation and activation of 5-lipoxygenase
- Up-regulation of pro-inflammatory cytokines like TNF-α and IL1-β
- Up-regulation of inflammatory mediators like LTB₄ and PGE₂

Therefore, it is worthy to use phytomedicines that have effects on cytokines and arachidonic acid metabolism is potential agents of preventing inflammation and thus chronic diseases.

An extensive literature search revealed that the leaves of *Annona squamosa*, *Annona reticulata* and *Annona muricata* are being used for medicinal applications for healing various ailments as traditional medicine practiced in tribal population of India. *Annona squamosa*, *Annona reticulata* and *Annona muricata* were collected from the Kondapalli reserve forest area, Vijayawada, Krishna District, Andhra Pradesh, India. The leaves of the plant were being used for investigation. Besides, young and fully expanded leaves were collected in the winter season morning time. The taxonomic identification of the above plants confirmed by Prof. S. M. Khasim, Department of Botany, Acharya Nagarjuna University, Guntur and Andhra Pradesh has been deposited in Botany Department, Acharya Nagarjuna University.
To investigate the scientific basis of the medicinal applications of *Annona squamosa*, *Annona reticulata* and *Annona muricata* leaves, the powdered died leaves were extracted in various solvents like hexane, ethyl acetate, methanol aqueous methanol and water; aiming at to reveal their anti-inflammatory and anti-oxidant activities along safety studies.

Phytochemical screening of the crude extracts of all the three plants were performed in order to characterize the class of compounds which are present in the leaves. This qualitative screening included the tests for phytosterols, triterpenoids, glycosides, tannins, alkaloids, saponins, flavonoids, phenols, carbohydrates, proteins and lipids. The quantitative screening included the tests for phenolic and flavonoids.  

*In vitro* screening of the three plants extracts were analysed by antioxidant, anti-inflammatory, cytotoxicity and anti-diabetic properties. Antioxidant properties were screened by DPPH, superoxide, ABTS, DMPD free radical scavenging and ferric reducing antioxidant power (FRAP) assay. Anti-inflammatory properties were screened by 5-LOX inhibitory assay and LPS Induced cytokine (TNF-α) production in THP-1 monocytic Cells model. Cytotoxicity was screened by brine shrimp lethality assay. Anti-diabetic properties were screened by α-Amylase and α-Glucosidase enzyme inhibition assays. Based on this *in vitro* analysis the bioactive potent fraction was selected from each plant.

The bioactive guided potent fractions of three plants were screened for it’s safety by acute oral toxicity, micronucleus test and chromosomal aberration assay. These potent fractions were screened *in vivo* models for their therapeutic activities. As common symptom of pyrexia in inflammatory conditions, the antipyretic activity was estimated by brewer’s yeast induced pyrexia in rat model. Acute anti-inflammatory effect was assessed
by carrageenan induced anti-inflammatory model in rat, egg albumin induced anti-inflammatory model in rat and xylene induced ear oedema model in mice. Chronic anti-inflammatory effect was evaluated by adjuvant induced arthritis model in rat, by assessing paw volume, biochemistry, hematology, Serum TNF-α, IL-1β, PGE₂ and LTB₄ Profile, MDA and antioxidants profile in liver lysates, Histopathology and radiographic analysis.

This research work explains the outcome of phytochemical fractions of *Annona squamosa*, *Annona reticulata* and *Annona muricata* leaves selected for the screening of *in vitro* and *in vivo* anti-inflammatory and antioxidant activities along safety assessment.