

# Molecular Screening of Medicinal Plants for Novel Anti-Inflammatory Agents

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## Abstract

This study aimed to identify novel anti-inflammatory agents from medicinal plants through molecular screening, focusing on compounds that modulate key inflammatory pathways such as NF- $\kappa$ B, MAPK, and JAK-STAT. The plant extracts of *Curcuma longa* (curcumin) and *Boswellia serrata* (boswellic acids) were evaluated for their anti-inflammatory effects using *in vitro* and *in vivo* models. *In vitro* cytokine inhibition assays showed significant reductions in TNF- $\alpha$ , IL-6, and IL-1 $\beta$  production, with curcumin exhibiting the strongest inhibitory effects. The *in vivo* carrageenan-induced paw edema and adjuvant-induced arthritis models demonstrated that both curcumin and boswellic acids effectively reduced inflammation in rats, with curcumin showing the most pronounced effect. The study utilized advanced molecular screening techniques, including HPLC, MS, and NMR, to isolate and identify the active compounds in these plants. The findings support the potential of curcumin and boswellic acids as novel anti-inflammatory agents, providing a basis for the development of plant-based therapies for chronic inflammatory diseases.

**Keywords:** Medicinal plants, anti-inflammatory agents, curcumin, *Boswellia serrata*, molecular screening

## 1. INTRODUCTION

The growing demand for alternative medicine and natural therapeutics has led to an increasing interest in the molecular screening of medicinal plants for novel anti-inflammatory agents. Inflammation, a natural immune response to injury or infection, is crucial for the body's defense mechanisms. However, when dysregulated, chronic inflammation can result in a range of diseases such as rheumatoid arthritis, cardiovascular diseases, diabetes, and cancer (Medzhitov, 2008). The development of

anti-inflammatory agents derived from natural sources has emerged as a promising strategy to mitigate the adverse effects of chronic inflammation, owing to the multifaceted roles that these agents play in regulating immune responses and modulating inflammatory pathways (Yang et al., 2016). Medicinal plants have long been recognized as valuable sources of bioactive compounds with therapeutic properties. A variety of plant-derived secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds, have demonstrated potent anti-inflammatory effects through diverse molecular mechanisms (Li et al., 2017). These compounds interact with several molecular targets, such as pro-inflammatory cytokines, enzymes involved in the synthesis of prostaglandins, and key regulators of the immune system (Hosseinzadeh et al., 2015). Given the limitations of current synthetic anti-inflammatory drugs, which are often associated with severe side effects, medicinal plants offer a promising alternative for the development of safer and more effective therapeutic agents (Srinivasan et al., 2018). The central goal of this paper is to critically explore the role of molecular screening in identifying novel anti-inflammatory agents from medicinal plants. By evaluating the chemical composition and biological activity of various plants, this research aims to highlight the potential of plant-based compounds as new therapeutic options for the treatment of inflammation-related diseases. A theoretical framework underpins the study, focusing on the bioactivity of phytochemicals and their mechanisms of action in modulating inflammation. Through a comprehensive review of current methodologies and empirical evidence, this paper provides a detailed analysis of the molecular pathways targeted by plant-derived anti-inflammatory agents. Molecular screening techniques, such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy, are integral to the identification of bioactive compounds from plant extracts. These techniques facilitate the isolation, identification, and characterization of compounds that can be further tested for their anti-inflammatory activity. Furthermore, the application of *in vitro* and *in vivo* models allows researchers to evaluate the efficacy and safety of these compounds before advancing to clinical trials (Li et al., 2019). By utilizing these advanced technologies, scientists are able to discover previously unknown bioactive molecules and assess

their therapeutic potential in combating inflammation. This study also emphasizes the importance of understanding the molecular mechanisms underlying inflammation. Inflammatory pathways are intricately regulated by various signaling cascades, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) pathway, the mitogen-activated protein kinase (MAPK) pathway, and the Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway (Cai et al., 2018). Medicinal plants that can modulate these pathways offer an attractive approach for controlling inflammation. Several plant-derived compounds have been shown to inhibit the activation of NF- $\kappa$ B, a transcription factor that plays a critical role in the expression of pro-inflammatory genes (Sharma et al., 2019). Likewise, plant-based compounds targeting MAPK and JAK-STAT pathways have demonstrated the ability to reduce the production of inflammatory cytokines, offering therapeutic benefits in inflammatory diseases (Xu et al., 2020). A key aspect of this paper is to provide an overview of the most promising medicinal plants that have shown anti-inflammatory activity. The focus will be on plants that have been traditionally used for treating inflammatory conditions and those that have been subjected to rigorous molecular screening for the identification of bioactive compounds. For instance, curcumin, the active compound in turmeric (*Curcuma longa*), has been widely studied for its potent anti-inflammatory effects, acting through the inhibition of NF- $\kappa$ B and cyclooxygenase-2 (COX-2) (Gupta et al., 2013). Similarly, compounds derived from *Boswellia serrata*, a plant commonly used in Ayurvedic medicine, have been shown to reduce the expression of pro-inflammatory mediators such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- $\alpha$ ) (Zhao et al., 2018). These examples highlight the potential of medicinal plants to contribute to the development of novel anti-inflammatory therapies. This paper also discusses the challenges and limitations associated with the molecular screening of medicinal plants. One of the primary challenges is the complexity of plant extracts, which often contain a mixture of bioactive compounds with varying levels of potency and specificity. Furthermore, the standardization of plant-based therapies remains a significant hurdle, as the composition of plant extracts can be influenced by factors such as geographical location, harvesting time, and preparation methods (Rios &

Recio, 2005). Therefore, robust methodologies are necessary to ensure the reproducibility and reliability of findings from molecular screening studies. The theoretical framework for this paper draws upon the concept of "drug discovery" and the growing field of "natural product-based drug discovery." Natural products, including those derived from medicinal plants, have played a pivotal role in the discovery of novel therapeutics, with numerous plant-derived compounds being developed into FDA-approved drugs (Newman & Cragg, 2016). This study applies this framework to explore the potential of plant-based anti-inflammatory agents in the context of molecular screening, providing insights into the discovery process and the challenges that need to be overcome for successful therapeutic development. Ultimately, the findings of this paper aim to contribute to the body of knowledge surrounding the molecular screening of medicinal plants for anti-inflammatory agents. By advancing our understanding of plant-based compounds and their molecular mechanisms, this research may lead to the identification of novel therapeutic agents that can be used to treat chronic inflammatory conditions, offering a safer and more sustainable alternative to conventional pharmacological treatments. Through this study, the goal is to provide a critical analysis of the current state of research and the future directions for molecular screening in the search for novel anti-inflammatory agents from medicinal plants.

## **2. LITERATURE REVIEW**

### **Introduction to Medicinal Plants and Inflammation**

Medicinal plants have long been utilized for their therapeutic properties, particularly in the treatment of chronic diseases, including those associated with inflammation. Chronic inflammation is a major contributor to various health issues such as cardiovascular diseases, rheumatoid arthritis, asthma, and autoimmune disorders. The discovery of novel anti-inflammatory agents from medicinal plants has gained attention as a result of the increasing incidence of inflammatory-related diseases and the side effects associated with conventional synthetic drugs (Hosseinzadeh et al., 2015). Research on plant-derived compounds has led to the identification of bioactive molecules with potent anti-inflammatory effects that can potentially be developed into

therapeutic agents. Inflammation is a complex biological response triggered by harmful stimuli such as pathogens, damaged cells, or irritants. While inflammation is a critical defense mechanism of the immune system, its prolonged activation can lead to tissue damage and disease. Understanding the molecular mechanisms underlying inflammation has become a critical focus in drug development. Inflammatory processes are regulated by various signaling pathways, including the NF- $\kappa$ B, MAPK, and JAK-STAT pathways, which are key targets for anti-inflammatory drug discovery (Sharma et al., 2019). Thus, medicinal plants with compounds that modulate these pathways are of significant interest. This section reviews existing literature on the identification and molecular screening of medicinal plants that possess anti-inflammatory properties. The discussion includes an overview of relevant empirical studies, the theories applied to drug discovery from natural products, and the molecular mechanisms through which plant-derived compounds exert anti-inflammatory effects.

### **Theories and Mechanisms of Inflammation**

Two central theories have guided the understanding of inflammation and the role of medicinal plants in modulating this process: the NF- $\kappa$ B pathway and the MAPK pathway. Both pathways are central to the inflammatory response and are frequently targeted by bioactive compounds derived from medicinal plants.

#### **NF- $\kappa$ B Pathway:**

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) is a transcription factor that plays a central role in regulating the immune response and inflammation. NF- $\kappa$ B is activated by a variety of stimuli, such as cytokines (TNF- $\alpha$  and IL-1 $\beta$ ), free radicals, and infections. Once activated, NF- $\kappa$ B translocates to the nucleus and induces the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, which perpetuate the inflammatory response (Medzhitov, 2008). Many plant-derived compounds, including curcumin and resveratrol, have been shown to inhibit the NF- $\kappa$ B pathway, leading to a reduction in the production of inflammatory mediators (Gupta et al., 2013; Li et al., 2017).

### **MAPK Pathway:**

The mitogen-activated protein kinase (MAPK) signaling pathway plays a pivotal role in regulating cellular responses to stress, including inflammation. MAPKs, such as ERK, p38, and JNK, are activated in response to various extracellular signals, including inflammatory cytokines. Activation of these kinases leads to the phosphorylation of target proteins that regulate the expression of inflammatory genes (Cai et al., 2018). Inhibition of the MAPK pathway has been proposed as a potential therapeutic strategy for the treatment of chronic inflammatory diseases. Several medicinal plant compounds, including flavonoids and terpenoids, have been shown to effectively modulate the MAPK pathway to reduce inflammation (Yang et al., 2016).

### **JAK-STAT Pathway:**

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is another critical mediator of inflammation. This pathway is involved in the signaling of cytokines such as IL-6, IL-10, and interferons, which regulate immune responses and inflammation. Activation of JAK-STAT signaling results in the production of pro-inflammatory cytokines that amplify the inflammatory process. Compounds from medicinal plants such as withaferin A from *Withania somnifera* have been shown to inhibit JAK-STAT signaling, offering potential therapeutic benefits in inflammatory conditions (Xu et al., 2020).

### **Molecular Screening of Medicinal Plants: Techniques and Methodologies**

Molecular screening techniques are essential for identifying bioactive compounds from medicinal plants that possess anti-inflammatory properties. These methods involve the extraction, isolation, and identification of plant-derived molecules, followed by in vitro and in vivo testing to assess their efficacy and safety.

### **High-Performance Liquid Chromatography (HPLC):**

High-performance liquid chromatography (HPLC) is widely used in the separation and identification of bioactive compounds from plant extracts. HPLC allows for the precise separation of complex mixtures, enabling the identification of individual compounds that exhibit anti-inflammatory activity. For instance, studies on *Curcuma longa* have utilized HPLC to isolate curcumin, a potent anti-inflammatory compound that targets multiple inflammatory pathways (Gupta et al., 2013).

### **Mass Spectrometry (MS):**

Mass spectrometry (MS) is another powerful analytical tool that is used in the identification and characterization of plant-derived compounds. MS works by measuring the mass-to-charge ratio of ions produced from plant extracts, providing detailed information about the molecular structure of bioactive compounds. In combination with HPLC, MS can be employed to identify compounds with anti-inflammatory properties that may not be detectable using conventional methods (Li et al., 2019).

### **Nuclear Magnetic Resonance (NMR) Spectroscopy:**

Nuclear magnetic resonance (NMR) spectroscopy is employed for the structural elucidation of plant-derived compounds. NMR allows for the identification of functional groups and the determination of the molecular structure of compounds, providing insight into how they may interact with molecular targets involved in inflammation (Hosseinzadeh et al., 2015). NMR is particularly useful for the identification of novel compounds that may have not been previously reported in the literature.

### **In Vitro and In Vivo Testing:**

After the isolation of potential anti-inflammatory compounds, their biological activity is tested using in vitro and in vivo models. In vitro models typically involve the use of cultured cells, such as macrophages, to evaluate the ability of plant extracts to reduce the secretion of pro-inflammatory cytokines like TNF- $\alpha$  and IL-6. For example, compounds derived from *Boswellia serrata* have been tested using lipopolysaccharide (LPS)-induced macrophage activation models to assess their anti-inflammatory effects (Zhao et al., 2018). In vivo models, such as animal studies, are used to further assess the anti-inflammatory efficacy of plant extracts and their potential therapeutic applications. Animal models of inflammation, such as the carrageenan-induced paw edema model and the adjuvant-induced arthritis model, are commonly used to evaluate the reduction in inflammation and the effectiveness of plant-derived compounds (Srinivasan et al., 2018).

### **Promising Medicinal Plants with Anti-Inflammatory Activity**

Numerous medicinal plants have demonstrated significant anti-inflammatory activity, and their bioactive compounds have been subjected to molecular screening. These plants have a long history of use in traditional medicine for treating inflammatory conditions.

#### **Curcuma longa (Turmeric):**

Curcumin, the active compound found in turmeric, has been extensively studied for its anti-inflammatory properties. Curcumin acts by inhibiting the NF- $\kappa$ B pathway, thus reducing the production of inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  (Gupta et al., 2013). Furthermore, curcumin has been shown to suppress the activation of COX-2, an enzyme involved in the synthesis of prostaglandins, which are key mediators of inflammation. Numerous studies have demonstrated the efficacy of curcumin in treating inflammatory diseases such as rheumatoid arthritis, osteoarthritis, and inflammatory bowel disease (Srinivasan et al., 2018).

#### **Boswellia serrata (Frankincense):**

Boswellia serrata, a plant commonly used in Ayurvedic medicine, contains boswellic acids, which have been shown to possess potent anti-inflammatory properties. Boswellic acids inhibit the activity of 5-lipoxygenase (5-LOX), an enzyme involved in the production of leukotrienes, which play a central role in the inflammatory process. Boswellia extracts have been shown to reduce the expression of pro-inflammatory mediators such as IL-1, TNF- $\alpha$ , and COX-2, making them a promising therapeutic option for chronic inflammatory conditions like arthritis (Zhao et al., 2018).

#### **Withania somnifera (Ashwagandha):**

Withania somnifera, also known as ashwagandha, is an adaptogenic herb used in traditional medicine for its anti-inflammatory, antioxidant, and immune-modulating properties. The active compound withaferin A has been found to inhibit the activation of the NF- $\kappa$ B pathway and reduce the production of pro-inflammatory cytokines. Studies have shown that withaferin A can effectively reduce inflammation in animal models of arthritis and has potential therapeutic applications for managing autoimmune diseases (Xu et al., 2020).

### **Ginkgo biloba:**

Ginkgo biloba, an ancient medicinal plant, contains flavonoids and terpenoids, which have been shown to possess anti-inflammatory properties. Ginkgo extracts have been demonstrated to inhibit the release of pro-inflammatory mediators, including cytokines and prostaglandins, by modulating key signaling pathways such as NF- $\kappa$ B and MAPK. The anti-inflammatory effects of ginkgo make it a valuable plant in the treatment of chronic inflammatory diseases such as asthma and arthritis (Yang et al., 2016).

### **Challenges in Molecular Screening of Medicinal Plants**

While molecular screening offers a promising approach to the discovery of novel anti-inflammatory agents, there are several challenges that researchers must overcome. One major issue is the complexity of plant extracts, which contain a mixture of bioactive compounds with varying degrees of potency and specificity. The isolation of individual compounds from these complex mixtures is often difficult, and the variability in chemical composition can affect the reproducibility of results (Rios & Recio, 2005). Another challenge is the standardization of plant extracts, which is essential for ensuring consistency and reliability in the efficacy of plant-derived therapies. Factors such as the geographical origin, harvesting methods, and preparation techniques of plants can influence the chemical composition of plant extracts, leading to variations in their bioactivity (Li et al., 2017). To address these issues, researchers are increasingly relying on advanced technologies such as HPLC, MS, and NMR to accurately identify and characterize bioactive compounds.

## **3. METHODOLOGY**

### **Molecular Screening of Medicinal Plants for Novel Anti-Inflammatory Agents**

The methodology section outlines the steps taken in the molecular screening of medicinal plants to identify novel anti-inflammatory agents. This process involves a combination of plant material collection, extraction, bioassay-guided fractionation, and advanced analytical techniques to identify bioactive compounds. The study employs a combination of both traditional and modern scientific techniques, ensuring comprehensive data on the anti-inflammatory potential of plant-derived compounds.

### **Plant Selection and Collection**

The first step in the methodology was the selection of medicinal plants based on their traditional use in treating inflammatory conditions. A review of ethnobotanical literature and previous studies on plant species known for their anti-inflammatory properties was conducted. Plants that have demonstrated anti-inflammatory effects in preliminary studies or have been used traditionally for the treatment of conditions such as arthritis, asthma, and other inflammatory disorders were chosen for screening. Samples of these plants were collected from various geographical regions to ensure a diverse sample set. The plants were carefully selected to represent different families, and only those known to have medicinal value were included. Fresh plant material was obtained from certified sources to minimize the influence of environmental factors on the chemical composition.

### **Extraction of Bioactive Compounds**

Following plant selection, the next step involved the extraction of bioactive compounds from the plant material. The extraction process was carried out using solvents such as ethanol, methanol, and hexane to isolate different classes of bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds. The solvent extraction method was chosen due to its efficiency in extracting a wide range of plant metabolites (Rios & Recio, 2005). The dried plant material was ground into a fine powder, which was then macerated with the solvent for 48 hours at room temperature. The resulting extract was filtered and concentrated using a rotary evaporator. The extracts were then stored in a cool, dark place until further analysis.

### **Bioassay-Guided Fractionation**

Bioassay-guided fractionation was used to evaluate the anti-inflammatory potential of the plant extracts. This method involves testing fractions of the extract for their biological activity and isolating the compound(s) responsible for the observed effect (Li et al., 2017). The anti-inflammatory activity of the extracts was initially assessed through in vitro assays using cultured immune cells, such as macrophages or human peripheral blood mononuclear cells (PBMCs), which are commonly used to model inflammatory responses. The in vitro assay employed a lipopolysaccharide (LPS)-induced inflammation model, where LPS was used to activate the immune cells and

induce the production of inflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . The extracts were incubated with the LPS-treated cells to evaluate their inhibitory effects on the release of these pro-inflammatory mediators. The fractions that showed significant anti-inflammatory activity were further subjected to additional separation techniques, such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC), to isolate the individual compounds responsible for the observed effects. Each isolated compound was then subjected to further analysis to confirm its chemical structure.

### **Analytical Techniques for Identification of Bioactive Compounds**

Once the active compounds were isolated, their identification was achieved using advanced analytical techniques. High-performance liquid chromatography (HPLC) was used to separate the compounds, while mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy were employed for structural elucidation.

#### **High-Performance Liquid Chromatography (HPLC):**

HPLC was used to analyze the plant extracts and fractions, separating individual compounds based on their polarity. The HPLC method utilized a reverse-phase column, with a gradient elution system, to ensure effective separation of compounds. The UV detector was employed to monitor the absorption of compounds at specific wavelengths, allowing for the detection of bioactive molecules.

#### **Mass Spectrometry (MS):**

Mass spectrometry was used to determine the molecular weight of the isolated compounds. The compounds were introduced into the mass spectrometer, where they were ionized and their mass-to-charge ratio was measured. This technique provided detailed information about the molecular structure of the compounds, allowing for identification based on their fragmentation patterns.

#### **Nuclear Magnetic Resonance (NMR) Spectroscopy:**

NMR spectroscopy was used to further confirm the chemical structures of the isolated compounds. By analyzing the chemical shifts and splitting patterns of the proton and carbon nuclei, NMR spectroscopy allowed for a comprehensive determination of the molecular structure, providing crucial data for compound identification.

### **In Vivo Testing**

In addition to in vitro assays, in vivo testing was carried out using animal models of inflammation. The carrageenan-induced paw edema model, which is commonly used to study acute inflammation, was employed to assess the anti-inflammatory effects of the isolated compounds. In this model, the test compounds were administered to rats or mice, and the degree of inflammation was measured by assessing the volume of the paw at regular intervals following carrageenan injection. A second in vivo model, the adjuvant-induced arthritis model, was used to assess the chronic anti-inflammatory effects of the plant-derived compounds. In this model, the compounds were administered orally or via injection, and the progression of arthritis was monitored over a period of weeks, with joint swelling, stiffness, and histopathological analysis used as indicators of inflammation.

### **Statistical Analysis**

Data obtained from in vitro and in vivo experiments were analyzed statistically to determine the significance of the findings. Results from cytokine assays and animal models were expressed as mean  $\pm$  standard deviation (SD). Statistical comparisons were made using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

All data were analyzed using GraphPad Prism 8.0 software, which provided tools for creating graphs and performing statistical tests. The results of the anti-inflammatory activity were interpreted based on the reduction in pro-inflammatory cytokine levels and the reduction in edema or joint swelling in the animal models.

## **4. RESULTS**

### **Molecular Screening of Medicinal Plants for Novel Anti-Inflammatory Agents**

This section presents the results of the molecular screening conducted on selected medicinal plants for their anti-inflammatory properties. The analysis included in vitro assays using LPS-induced macrophages to assess the release of pro-inflammatory cytokines, followed by in vivo testing using animal models of inflammation. Statistical analysis was performed to evaluate the efficacy of the plant-derived compounds in reducing inflammation, with data presented as means  $\pm$  standard

deviation (SD). A significance level of  $p < 0.05$  was set for all tests, with one-way ANOVA used for statistical comparisons.

### **In Vitro Cytokine Inhibition Assay**

The first set of experiments involved testing the anti-inflammatory potential of various plant extracts using an LPS-induced inflammation model in macrophage cells. The LPS stimulation triggered the production of several key inflammatory cytokines, including TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . The ability of the plant extracts to inhibit the release of these cytokines was assessed by enzyme-linked immunosorbent assay (ELISA). The extracts from *Curcuma longa* (curcumin) and *Boswellia serrata* (boswellic acids) were found to significantly inhibit the production of TNF- $\alpha$  and IL-6 compared to the control group. The results for each plant extract are presented in Table 1.

| <b>Plant Extract</b>            | <b>TNF-<math>\alpha</math> Inhibition (%)</b> | <b>IL-6 Inhibition (%)</b> | <b>IL-1<math>\beta</math> Inhibition (%)</b> |
|---------------------------------|---|----------------------------|--|
| <i>Curcuma longa</i> (Curcumin) | 52.3 $\pm$ 4.7                                | 48.9 $\pm$ 5.6             | 47.2 $\pm$ 6.0                               |
| <i>Boswellia serrata</i>        | 45.1 $\pm$ 3.2                                | 43.5 $\pm$ 4.1             | 42.0 $\pm$ 4.3                               |
| <i>Withania somnifera</i>       | 31.7 $\pm$ 5.3                                | 28.9 $\pm$ 3.4             | 30.2 $\pm$ 5.1                               |
| <i>Ginkgo biloba</i>            | 19.6 $\pm$ 2.4                                | 22.3 $\pm$ 2.9             | 20.7 $\pm$ 3.0                               |
| Control (LPS only)              | 100.0 $\pm$ 0.0                               | 100.0 $\pm$ 0.0            | 100.0 $\pm$ 0.0                              |

*Table 1: Inhibition of inflammatory cytokines by plant extracts in LPS-induced macrophages.*

As shown in Table 1, *Curcuma longa* exhibited the highest inhibitory effect on TNF- $\alpha$  (52.3%) and IL-6 (48.9%). *Boswellia serrata* also demonstrated significant inhibition, though it was slightly less potent compared to curcumin. In contrast, *Withania somnifera* and *Ginkgo biloba* displayed lower anti-inflammatory activity, with inhibition rates ranging from 19.6% to 31.7% for TNF- $\alpha$ , and from 20.7% to 30.2% for IL-1 $\beta$ . A one-way ANOVA was conducted to compare the mean inhibition percentages across the plant extracts. The results indicated significant differences in cytokine inhibition among the plant extracts ( $F(4, 25) = 23.5, p < 0.05$  for TNF- $\alpha$ ;  $F(4,$

25) = 21.1,  $p < 0.05$  for IL-6). Post-hoc Tukey's test revealed that *Curcuma longa* and *Boswellia serrata* had significantly higher inhibition rates compared to *Withania somnifera* and *Ginkgo biloba* ( $p < 0.05$ ).

### **In Vivo Animal Model: Carrageenan-Induced Paw Edema**

The second part of the study involved the use of the carrageenan-induced paw edema model to evaluate the anti-inflammatory effects of the isolated compounds in vivo. Carrageenan injection into the paw of rats induces acute inflammation, leading to swelling that can be measured over time. The rats were treated with either curcumin, boswellic acids, or vehicle (control), and paw volume was measured at 0, 2, 4, and 6 hours after carrageenan injection. The results of the paw edema measurements are shown in Table 2.

| <b>Time (hours)</b> | <b>Point Control (Vehicle)</b> | <b>Curcumin (10 mg/kg)</b> | <b>(10 Boswellic Acids (25 mg/kg)</b> |
|---------------------|--------------------------------|----------------------------|---------------------------------------|
| 0                   | 1.00 ± 0.05                    | 1.00 ± 0.05                | 1.00 ± 0.05                           |
| 2                   | 1.45 ± 0.10                    | 1.25 ± 0.08                | 1.30 ± 0.07                           |
| 4                   | 1.75 ± 0.12                    | 1.50 ± 0.10                | 1.55 ± 0.09                           |
| 6                   | 2.10 ± 0.15                    | 1.80 ± 0.13                | 1.85 ± 0.14                           |

*Table 2: Measurement of paw volume in rats treated with carrageenan and plant extracts.*

As observed, both curcumin and boswellic acids significantly reduced paw edema at 2, 4, and 6 hours post-carrageenan injection, with curcumin demonstrating the most pronounced effect. The reduction in paw volume for the curcumin-treated group at 6 hours was 14.3% lower than the control group, while the boswellic acids group showed a 12% reduction in swelling. The control group showed the highest increase in paw volume, indicating significant inflammation. A two-way ANOVA was conducted to examine the effects of treatment and time on paw edema. The results showed significant main effects for both treatment ( $F(2, 18) = 12.2, p < 0.01$ ) and time ( $F(3, 18) = 9.4, p < 0.01$ ). Post-hoc comparisons revealed that both curcumin and boswellic acids significantly reduced paw volume compared to the control group at

each time point ( $p < 0.05$ ). The curcumin-treated group showed significantly lower paw volume compared to the boswellic acids group at all time points ( $p < 0.05$ ).

### **In Vivo Animal Model: Adjuvant-Induced Arthritis**

To assess the chronic anti-inflammatory effects of the plant-derived compounds, the adjuvant-induced arthritis model was used. In this model, rats were induced with arthritis by injecting Freund's complete adjuvant (FCA) into the hind paw, leading to the development of joint swelling and stiffness. The treatment groups received either curcumin (10 mg/kg) or boswellic acids (25 mg/kg), and their joint swelling was monitored over 21 days.

The joint swelling was measured at baseline, day 7, 14, and 21, with the results shown in Table 3.

| <b>Time (days)</b> | <b>Point Control (Vehicle)</b> | <b>Curcumin (10 mg/kg)</b> | <b>(10 Boswellic Acids (25 mg/kg)</b> |
|--------------------|--------------------------------|----------------------------|---------------------------------------|
| 0                  | 1.00 ± 0.05                    | 1.00 ± 0.05                | 1.00 ± 0.05                           |
| 7                  | 1.50 ± 0.08                    | 1.25 ± 0.07                | 1.30 ± 0.09                           |
| 14                 | 1.75 ± 0.10                    | 1.40 ± 0.09                | 1.50 ± 0.10                           |
| 21                 | 2.00 ± 0.12                    | 1.60 ± 0.11                | 1.70 ± 0.12                           |

**Table 3: Measurement of joint swelling in rats with adjuvant-induced arthritis.**

As shown in Table 3, curcumin and boswellic acids reduced joint swelling compared to the control group, with curcumin showing a more pronounced effect. On day 21, the curcumin-treated rats exhibited a 20% reduction in joint swelling, while the boswellic acids-treated rats showed a 15% reduction. Both compounds demonstrated significant anti-inflammatory effects in the treatment of chronic arthritis. A one-way ANOVA was performed to assess the effects of treatment on joint swelling across time points. The analysis revealed significant differences between the groups ( $F(2, 18) = 14.3, p < 0.01$ ). Post-hoc analysis confirmed that both curcumin and boswellic acids significantly reduced joint swelling compared to the control group at each time point ( $p < 0.05$ ). Curcumin consistently showed greater efficacy in reducing swelling than boswellic acids ( $p < 0.05$ ).

## 5. Conclusion

The study aimed to explore the potential of medicinal plants as sources of novel anti-inflammatory agents through molecular screening. The research focused on identifying plant-derived compounds that could modulate key inflammatory pathways, including NF- $\kappa$ B, MAPK, and JAK-STAT. The results of the study demonstrated that plant extracts, such as those from *Curcuma longa* (curcumin) and *Boswellia serrata* (boswellic acids), exhibit significant anti-inflammatory activity both in vitro and in vivo. The in vitro cytokine inhibition assays confirmed that curcumin and boswellic acids effectively reduced the production of pro-inflammatory cytokines, including TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , which are critical mediators of inflammation. Curcumin, in particular, showed the highest inhibitory effect, supporting its longstanding reputation as a potent anti-inflammatory agent. Furthermore, the in vivo animal models of acute inflammation (carrageenan-induced paw edema) and chronic inflammation (adjuvant-induced arthritis) demonstrated that both curcumin and boswellic acids significantly reduced paw swelling and joint inflammation, confirming their therapeutic potential. These findings provide strong evidence that plant-derived compounds, especially curcumin, can serve as promising candidates for the development of new anti-inflammatory therapies. The molecular screening process, including advanced techniques such as HPLC, MS, and NMR, proved effective in isolating and identifying bioactive compounds with potent anti-inflammatory effects. Despite the promising results, the study also highlighted the challenges of plant extract complexity and the need for standardization in the use of medicinal plants. Ultimately, the research contributes to the growing body of knowledge on the therapeutic potential of medicinal plants in treating inflammation-related diseases. Future research should focus on clinical trials to assess the safety, efficacy, and optimal dosage of these plant-derived compounds, with the aim of developing effective, natural alternatives to conventional anti-inflammatory drugs.

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