In-Vitro Assay to Investigate the Anti-Inflammatory Activity of Hydroalcoholic Leaves Extract of *Acacia Auriculiformis* A.Cunn. Ex Benth.

Vaishnavi Nimbalkar*, Manish Kamble, Jagdish Baheti
Kamla Nehru College of Pharmacy, Butibori, Nagpur (M.S.) India
**Corresponding author*

Abstract: **Secondary metabolite obtained from medicinal plants are progressively used in the treatment of various diseases as a complementary medicine. When infectious microorganisms such as bacteria, viruses or fungi invade the body, reside in particular tissues and/or circulate in the blood, inflammation occurs and also happen in response to tissue injury, cell death, cancer, ischemia and degeneration. The present study reveals the scientific evidence to the traditional use of Earleaf acacia (*Acacia auriculiformis*) as anti-inflammatory drug. The HRBC membrane stabilization method has been used to study the invitro anti-inflammatory activity. The result expressed the concentration dependent membrain stabilizing potential.**

Keywords: **Earleaf acacia, anti-inflammatory, HRBC, stabilization**

I. INTRODUCTION

Inflammation is a defensive mechanism of our body to hazardous stimuli such as tissue injury or allergy. It is frequently associated with pain and involves occurrences of some events such as increase of vascular permeability, increase of protein denaturation and membrane alteration. These events usually occurs when infectious microorganisms such as bacteria, viruses or fungi invade the body, circulates in the blood[1] or some times in response to processes such as tissue injury, cell death, cancer, ischemia and degeneration[2].Pain, redness, warmness and swelling are the primary symptoms of inflammation. When there is an injury, blood circulation towards the injured tissue increases and this produces redness[3]. Inflammatory crisis can be controlled and supressed by various medicines. Some of them aresteroids, NSAID's, and immune suppressant's. Though, these are associated with many adverse effects, there is need to apply natural anti-inflammatory agents to increase pharmacological response and the lowest degree of unwanted side effects[4]. Herbal medicines are promoting as alternatives or as complimentary medication for allopathic medicines with no or less adverse effects. In the present study assessment of in-vitro antiinflamatory activity was studied using *Acaccia auriculiformis* leaves extract.

Acacia auriculiformis (Fabaceae) also known as Earleaf acacia or Pondicherry teak has its natural distribution worldwide. It is a straight, medium-sized evergreen tree reaches upto 35m heights[5]. *A. auriculiformis* displays axillary buds that can support multiple shoots[6]. Presence of dormant buds in the lower part of the stem shows the ability to coppice[7] to some extent. Mature leaves are dark green in colour with linear to very narrowly elliptic and falcate. The leaves are glabrous, 8–22.5 cm long (average 10–20 cm) and 10–52 mm wide (average 12–30 mm), generally clearly distinguished from the leaf margin and characterised by three prominent longitudinal nerves[8]. Between and mostly parallel to them appear many secondary nerves[9]. The tree is used as a folk medicine from long time. Decoction of root is used to treat aches and sore eyes while an infusion of bark is used to treat rheumatism[10]. Matured seeds are used traditionally to treat skin diseases like itching, allergy and rashes. The plant is useful as anti-malarial remedy[11]. It is reported that plant has shown various pharmacological activities like antioxidant[12], antimicrobial[13], antifilarial[14], cestocidal[15], hepatoprotective and antidiabetic[16], wound healing[17].

II. MATERIAL AND METHOD

Collection of Plant Materials

The leaves of *Acacia auriculiformis* were collected from college campus of Kamla Nehru College of Pharmacy, Butiburi, Nagpur, Maharashtra. Plant material was identified and authenticated in the department of Botany, RTM Nagpur University Nagpur, Maharashtra, with specimen no 10340. The collected materials were cleaned and dried for further process of extraction.

Preparation of the Extract

Leave was taken (100g) and subjected to hydro alcoholic extraction (Soxhlet Extraction). Then, it was filtered and evaporated under rotary vacuum evaporator until semi solid consistency is obtained. Then the aqueous extract was then re-dissolved in water at 1 mg/ml ratio and used for evaluating in-vitro anti-inflammatory-inflammatory potential.

In-vitro anti-inflammatory activity :

HRBC Membrane Stabilization

Human red blood cells (HRBC) suspension was prepared according to the previously described method[18]. The blood was sample was evacuated from healthy human volunteers who haven't undergone any medication of NSAIDs for at

least 2 weeks prior to the experiment. Then the blood sample was transferred to the centrifuge tubes and the tubes were centrifuged at 3000 rpm for 10 min. the sample was washed three times with equal volume of normal saline. The previously measured volume of blood was re-constituted as 10% v/v suspension with normal saline. Hypotonicity-induced haemolysis was used for membrane stabilization assay. The reaction mixture (4.5 ml) consisted of 2ml hypotonic saline (0.25% NaCl), 1 ml extract (200, 400, 600, 800 and 1000 µg/ml) in normal saline and 0.5 ml of 10% human RBC in normal saline. In blank 1 ml of isotonic saline was used instead of extract while control was devoid of red blood cells. The mixtures were incubated at 50^0C for 30 min. The tubes were centrifuged at 1500 rpm for 10 min after cooling the tubes under running tap water for 20 min. Absorbance of the supernatant was read at 560nm.

Membrane stabilization was calculated by using the formula-

(Abs of blank – Abs of extract) / Abs of control × 100.

The control represents 100%.

Drugs interact with membrane hence the model used in this study is Erythrocytes membrane stabilization[19-20]. NSAIDs stabilize erythrocytes against stress hemolysis. Moreover, they prevent the release of hemoglobin as a result of their membrane stabilizing activity[21]. The human red blood cells (HRBC) model is selected to assess the anti-inflammatory activity of *Acaciaauriculiformis*. In this study, 05 different concentration of aqueous extract of *Acaciaauriculiformis* leaves extract have been evaluated for their HRBC membrane stabilization activity. High concentration (1000 µg/ml) of the leaves extract was found to stabilize the HRBC membrane up to 28.23% (Table 1), which is comparable to the activity of the standard NSAID Aspirin (49.61%). Themembrane stabilization activity was observed in all the dilution of extract in a dose-dependent manner.

Table 1: HRBC membrane stabilization activity of the aqueous extract of *Acacia auriculiformis*

Concentration (µg/ml)	Stabilization (%)
200	10.42
400	15.71
600	22.54
800	28.23
1000	49.61
Aspirin (1000 µg/ml)	68.32

III. CONCLUSION

Present study deals with significant in-vitro study on anti-inflammatory potential of *Acacia auriculiformis* leaves. Results obtained from the present study provide scientific evidence for the use of this plant in folk medicine. Further, the present study suggests that *acacia auriculiformis* could serve as a lead in the development of a novel herbal anti-inflammatory agent. The study finding showed that the hydro alcoholic extract of *Acacia auriculiformis* possess anti-inflammatory activity. The inhibition is dose dependent with high doses of extract showing a significance percentage of inhibition. The anti-inflammatory effects exhibited by the extracts could as well be attributed to other phytochemicals present in the leaves. Past studies have shown that phytochemicals including tannins possess anti-inflammatory activity[22-23]. To better understand the mechanism by which phytochemicals of *Acacia auriculiformis* act, further studies are warranted.

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