# Preparation of *Camellia sinensis* (Green Tea) and *Acacia nilotica* (Babul) Herbal Formulation and its Anti-Inflammatory Activity

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# ABSTRACT

**Introduction:** Acacia nilotica is otherwise called Babul is multipurpose nitrogen fixing tree legume. Its roots are beneficial in treating tumors, cancer, and tuberculosis. One of the most consumed beverages in the world, green tea (*Camellia sinensis*) has been researched for its potential health benefits in treating conditions like cancer, diabetes, obesity, heart disease, and neurological illnesses. The aim of this present study is to assess the combined effects of green tea and babul herbal formulation as an anti-inflammatory agent. **Materials and Methods:** Green tea and Babul extract was prepared. Albumin denaturation assay and egg albumin denaturation assay were used to assess the anti-inflammatory activity. **Results:** Both assays showed that Green tea and babul extract was effective in inhibiting the albumin denaturation at different concentrations. **Conclusion:** We conclude that green tea and babul herbal formulation can be a potent anti-inflammatory agent.

Keywords: Green tea, Babul, Herbal extract, Anti-inflammatory activity.

# **INTRODUCTION**

Any live cell or tissue that has been injured or damaged will respond by becoming inflamed. Increased blood flow to the tissue results in an increase in temperature, redness, swelling, and pain. This is how it is identified. For many years, synthetic chemicals have been successfully employed to treat many ailments. Cancer and rheumatoid arthritis, periodontitis are some diseases caused by chronic inflammation. During the inflammation process, a variety of immune cells are drawn in, including neutrophils, monocytes, and macrophages. These immune cells then produce pro- and anti-inflammatory cytokines and enzymes, including tumour necrosis factor, interleukin, matrix metalloproteinases, etc. Nuclear factor -kappaß controls genes involved in a variety of inflammatory processes, many of which are important in modifying inflammatory and immunological responses.<sup>[1]</sup> Traditional plant-derived compounds have been utilised as medicine since ancient times, and they continue to



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play an essential role in health care, particularly in rural areas with limited access to modern medicine. Phytochemicals found in plants have been demonstrated to operate as defence systems against a variety of diseases.

Babul, also known as *Acacia nilotica*, is a versatile nitrogen-fixing tree legume. It grows from sea level to a height of nearly 2000 metres, enduring high temperatures (>50°C) and air aridity yet being vulnerable to ice when young. From Egypt to Mauritania southward to South Africa, it is widely distributed throughout subtropical and tropical Africa, while in Asia it is widespread eastward to Pakistan and India.<sup>[2]</sup> According to Kalaivani T and Mathew L.,<sup>[3]</sup> *A. nilotica* roots are beneficial in treating tumours, cancer, and tuberculosis. This tree's leaves and gum have also been demonstrated to be efficient antibacterial agents, with efficacy in the treatment of diarrhoea, wound dressing, and inflammation. These medicinal properties are attributed to the presence of cyclitols, fatty acids, secondary metabolites including amines and alkaloids etc.

One of the most consumed beverages in the world, green tea<sup>[5]</sup> has been researched for its potential health benefits in treating conditions like cancer, diabetes, obesity, heart disease, and neurological illnesses.<sup>[6]</sup> The principal polyphenol present in

green tea, which is made from the *Camellia sinensis* plant, is epigallocatechin gallate (EGCG). In numerous cell types, EGCG has been shown to have anti-inflammatory and antioxidant,<sup>[7]</sup> characteristics. EGCG is regarded to offer therapeutic value in a variety of inflammatory disorders, including atherosclerosis, arthritis, and dry eye disease.<sup>[8]</sup> There are studies which demonstrate anti-inflammatory<sup>[9]</sup> activity using green tea and babul as two separate compounds. Hence the aim of this present study is to assess the combined effects of green tea and babul herbal formulation as an anti inflammatory agent.

# **MATERIALS AND METHODS**

## **Preparation of the Sample**

Green tea and Babul extract was commercially obtained. 1 mg of dried Green tea and 1 mg of Babul powder was dispersed in 100 mL of distilled water and boiled for 10 min at 60-80°C. Whatman No.1 filter paper was used to filter the extract. The filtered solution was reheated for 20-30 min to reduce the 100ml solution into a 5ml solution of Green tea and Babul herbal solution.

## **Anti-inflammatory activity**

#### Albumin denaturation assay

The following convention, which Muzushima and Kabayashi established with particular modifications, was used to investigate the anti-inflammatory effect of green tea and babul. 0.45 mL of bovine serum albumin (1% aqueous solution) was mixed with 0.05 mL of green tea and babul of varied fixation (10, 20, 30, 40, and 50 L), and a small amount of 1N hydrochloric acid was used to adjust the pH to 6.3. These samples were heated to 55°C in a water bath for 30 min after being incubated at room temperature for 20 min. After cooling the samples, the absorbance at 660 nm was calculated spectrophotometrically. The standard was diclofenac sodium. The control used is DMSO.

Percentage of protein denaturation was determined utilizing following equation,

% inhibition= Absorbance of control- Absorbance of sample ×100

# Absorbance of control

#### Egg albumin denaturation assay

A 5ml solution was made which was comprised of 2.8ml of freshly prepared phosphate buffered saline of pH-6.3, 0.2 ml of egg albumin extracted from hens egg. Specific concentrations were prepared separately for green tea and babul as  $(10\mu L, 20\mu L, 30\mu L, 40\mu L, 50\mu L)$ . Diclofenac Sodium was used as the positive control. Then the mixtures were heated in a water bath at 37°C for 15 min. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

# **RESULTS AND DISCUSSION**

Protein denaturation causes protein molecules to lose their biological capabilities. Protein denaturation has been linked to the development of inflammatory diseases such cancer, diabetes, and rheumatoid arthritis. Therefore, a substance's ability to stop protein denaturation may also aid in stopping inflammatory illnesses. There are some disadvantages to using animals in experimental pharmacological research, including ethical issues and the absence of a justification for their use when better methods are available or can be investigated. As a result, for the current study's *in vitro* assessment of the anti-inflammatory effect of the aqueous extracts of green tea and babul, the protein denaturation bioassay was chosen.<sup>[10]</sup>

The present study results were observed in concentration dependent manner obtained from BSA assay (Figure 1). It was effective in inhibiting the protein denaturation at different concentrations. Maximum inhibition was observed at 20  $\mu$ L concentration where it showed higher activity than the standard drug. (Figure 2) shows the results obtained from egg albumin assay. The extract was effective in inhibiting the albumin denaturation at different concentrations. Maximum inhibition was observed at 10  $\mu$ L concentration where it showed higher activity than the standard drug.

Previous research on *Acacia nilotica*,<sup>[11]</sup> demonstrated that the methanol extract of the plant's pods had a considerable anti-inflammatory effect, with the tannin fraction of the extract being more effective than the methanol extract overall. A study conducted on the synergistic effect of chlorogenic acid (phenolic compound found in coffee) and l-theanine (amino acid found in green tea) showed that when compared to their individual effects, the combined actions of chlorogenic acid and L-theanine have a highly substantial effect.<sup>[12]</sup> The present study focused mainly on the combined effect of green tea and babul extract where it showed significant anti-inflammatory activity.



Figure 1: Graph showing the anti-inflammatory activity of green tea and babul herbal formulation through albumin denaturation assay. In this assay, bovine serum albumin is used as protein and is compared to the standard anti-inflammatory agent (i.e. Diclofenac sodium). It was effective in inhibiting the albumin denaturation at different concentrations. Maximum Inhibition was observed at 20 μL concentration which was more than 50%.



Figure 2: Graph showing the anti-inflammatory activity of green tea and babul herbal formulation through egg albumin denaturation assay. In this assay, egg albumin is used as protein and is compared to the standard anti-inflammatory agent (i.e. Diclofenac sodium). Maximum inhibition was observed at 10 μL which was more than 50%.

Due to its pleiotropic effects and multiple targets, green tea and EGCG may be used to improve the quality of life for those who suffer from inflammatory disorders. The dried pods of *A. nilotica* have an anti-inflammatory impact on both the exudative and proliferative phases of inflammation, which justifies its widespread usage in folk medicine to treat inflammation-related diseases.

# CONCLUSION

Within the limitations of the present study, we conclude that green tea and babul herbal formulation can be a potent anti-inflammatory agent. Studies to be undertaken comparing the combined and individual effects of green tea and babul extract. In order to identify the active components present in the extract and to pinpoint their precise mechanism of action, further research is necessary.

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# **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

### ABBREVIATIONS

EGCG: Epigallocatechin gallate; DMSO: Dimethyl sulfoxide.

#### **SUMMARY**

Camellia sinensis is a big evergreen shrub native to Eastern Asia that is widely grown. The prevention of various diseases and other significant health advantages have recently rekindled interest in green tea. Acacia nilotica, a significant ornamental and therapeutic plant native to tropical and subtropical areas and a member of the Fabaceae family (also known as babul), is a source of numerous active secondary metabolites that have the greatest chance of success in the near future as candidates for new drugs. Previous studies have shown the pharmacological and toxicological effects of green tea and babul on both animals and people, including reports of its anti-inflammatory qualities. Green tea and babul were commercially obtained and the herbal formulation was prepared and it was subjected to protein denaturation assay to assess the anti-inflammatory activity. The present study results showed that green tea and Babul herbal extract has a potent anti-inflammatory activity when compared to standard agent. As the concentration increased, the % of protein denaturation inhibition also increased.

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