

# In vitro protein denaturation inhibition assay of *Eucalyptus globulus* and *Glycine max* for potential anti-inflammatory activity

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## Introduction

Anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signaling to the brain.<sup>[1]</sup>

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation, and membrane alteration.<sup>[2,3]</sup> It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area.<sup>[4]</sup> Loss of function occurs depends on the site and extent of injury.<sup>[5]</sup> Inflammation usually occurs when infectious microorganisms such as bacteria, viruses, or fungi invade the body, reside in particular tissues

## ABSTRACT

**Background:** *Eucalyptus globulus* and *Glycine max* (G-max) are well known and widely used herbs, which contain several interesting bioactive constituents and possess health-promoting properties. To reduce the use of animals in conducting anti-inflammatory activity, *in vitro* studies as animal ethics is important as human welfare. **Aim:** The present work was carried out to evaluate the potential anti-inflammatory property of the combination effect of *E. globulus* + G-max extracts against the denaturation of protein *in vitro* using egg and bovine serum albumin (BSA) method. **Materials and Methods:** The test extract, at different concentrations, was incubated with egg and BSA under controlled experimental conditions and subjected to determination of absorbance at 660 nm to evaluate the anti-inflammatory property. Methotrexate was used as the reference standard drug. **Results:** The present results exhibited a concentration-dependent inhibition of protein (albumin) denaturation by the test extract. The outcome of methotrexate was found to be less effective when compared with the test extract. **Conclusion:** From the present findings, it can be concluded that the *E. globulus* + G-max possessed a potent anti-inflammatory effect against the denaturation of protein *in vitro*. This effect could be either due to the synergistic activity of these two herbal drug combinations.

**Keywords:** Anti-inflammatory potential, eucalyptus oil, glycine max, protein denaturation

and/or circulate in the blood.<sup>[6,7]</sup> At present, chronic inflammation is thought to be a risk factor for a broad range of age-related diseases such as hypertension, diabetes, atherosclerosis, and cancer.<sup>[8]</sup> There are several possible factors that initiate and maintain a low-grade inflammatory response. These include aging, unbalanced diet, low level of sex hormones, and smoking.<sup>[9]</sup> The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost.<sup>[10]</sup>

The rationale behind implementing this assay is that the denaturation of albumin protein leads to the formation of antigens which initiate type III hypersensitive reaction leading to inflammation.<sup>[11]</sup> Nowadays, methotrexate (Mtx) is the most commonly prescribed drug in inflammatory disorders. Hence, Mtx was used as a reference standard drug to perform this assay.

*Eucalyptus globules* is an evergreen tree native to Australia and a member of Myrtaceae family.<sup>[12]</sup> Eucalyptus oil (EO) is an essential oil and having potent anti-inflammatory action<sup>[13]</sup> [Figure 1]. The aromatic constituents of EO are used as analgesic, anti-inflammatory, and antipyretic remedies.<sup>[14]</sup> EO contains  $\alpha$ -pinene and 1,8-cineole and acts as an antioxidant, with strong radical scavenging activity.<sup>[15]</sup> Juergens

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et al. examined the role of eucalyptol as inhibitor of the production and synthesis of tumor necrosis factor- $\alpha$ , interleukin- $1\beta$ , leukotriene B $_4$ , and thromboxane B $_2$  in human blood monocytes, suggesting that eucalyptol is a strong inhibitor of cytokines that might be suitable for long-term treatment of airway inflammation in bronchial asthma and other steroid-sensitive disorders.<sup>[16]</sup>

The *Glycine max* (G-max) is an annual legume of the Fabaceae family.<sup>[16]</sup> It is indigenous to East Asia and China but now is extensively cultivated in many temperate regions of the world.<sup>[17]</sup> Conventionally, soybean has been an excellent source of proteins<sup>[18]</sup> [Figure 2]. Glyceollin on soybean can suppress inflammation by inhibiting the activation of nuclear factor-kappa (NF-kB), thus suppressing the increased production of pro-inflammatory cytokines.<sup>[19]</sup> Glyceollin on soybean can suppress inflammation by inhibiting the activation of NF-kB, thus suppressing the increased production of pro-inflammatory cytokines.<sup>[20]</sup>

Hence, turning to safe, effective, and time-tested ayurvedic herbal drug combination would be a preferable option and prime concerning the issue of our study.

## Materials and Methods

### Plant material

EO purchases from the local market. G-max is obtained from the soya bean seeds. Soya bean seeds purchase from the local market, then seeds are grind and then pass through sieve no. 85 and the powder of G-max were collected for the study.

### Chemicals

Methotrexate (Imutrex) obtained from Cipla company. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

### Evaluation of *in vitro* anti-inflammatory activity

#### Protein denaturation using egg albumin<sup>[21]</sup>

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 2 ml of varying concentrations of aqueous extract of *Eucalyptus globulus* and G-max (50:50 ratio) so that final concentrations become 100, 200, 400, 800, and 1000  $\mu\text{g}/\text{ml}$ . A similar volume of double-distilled water served as control. Then, the mixtures were incubated at  $37 \pm 2^\circ\text{C}$  in a BOD incubator for 15 min and then heated at  $70^\circ\text{C}$  for 5 min. After cooling, their absorbance was measured at 660 nm. Methotrexate was used as a reference standard drug.

### Formula

The percentage inhibition of protein denaturation was calculated using the following formula:

Percentage inhibition =  $(\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$ .

### Protein denaturation using bovine serum albumin (BSA)<sup>[22,23]</sup>

The reaction mixture was consisting of aqueous extract of *E. globulus* and G-max (50:50 ratio) at different concentrations and 1% of aqueous solution of bovine albumin. The samples were incubated at  $37^\circ\text{C}$  for 20 min and then heated at  $57^\circ\text{C}$  for 20 min. After cooling the samples, the absorbance of turbidity was measured at 660 nm.

### Formula

Percentage of inhibition of protein denaturation was calculated as follows:

Percentage inhibition =  $(\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$ .

## Results and Discussion

The effect of aqueous extract of *E. globulus* (EO) and G-max was evaluated against the denaturation of egg albumin and BSA. The results



Figure 1: Eucalyptus oil (*Eucalyptus globulus*) (<https://i.ytimg.com/vi/G7HaYzBj31c/maxresdefault.jpg>)

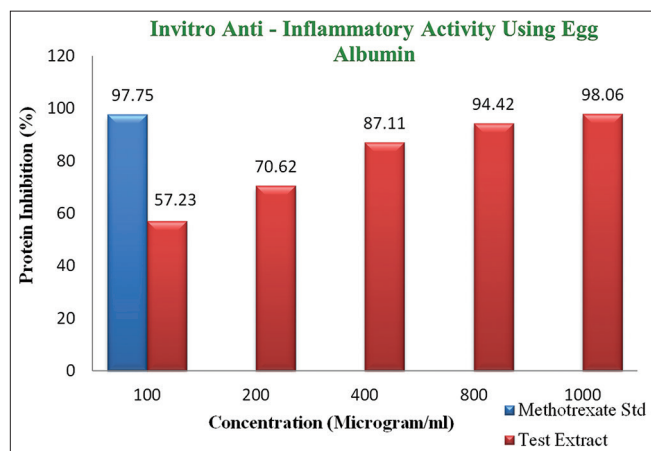


Figure 2: Soybean seeds (*Glycine max*) (<https://3.imimg.com/data3/IT/UK/MY-3451757/soybean-seed-500x500.jpg>)

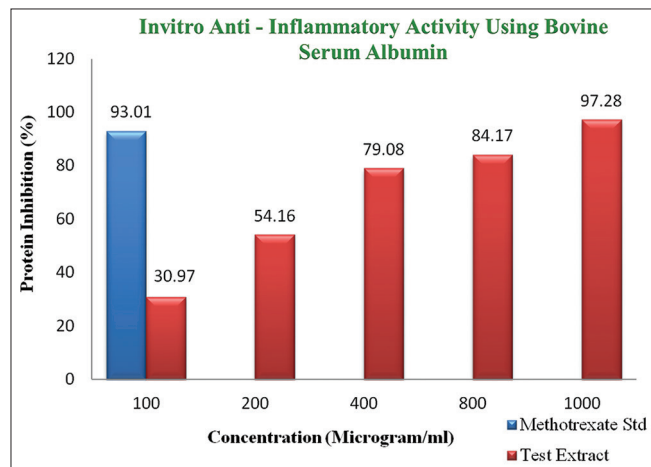
are summarized in Figures 3 and 4. The combination of herbal extract exhibited significant anti-inflammatory activity at 100–1000 µg/ml by protein denaturation inhibition. The effect of the herbal extract was studied by comparing with the standard methotrexate.

The autoantigen production in inflammation is due to denaturation of protein and several studies reveal that protein denaturation is one of the reasons for inflammation.<sup>[24]</sup> Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well-documented causes of inflammation.<sup>[25]</sup> The decrease in absorbance of the test sample with respect to control indicated stabilization of protein, i.e., inhibition of protein (albumin) denaturation or anti-denaturation effect by the test extract and the reference drug methotrexate.<sup>[26]</sup>

The herbal extract at a concentration (1000 µg/ml) showed the highest percentage inhibition, i.e., 98.06% in egg and 97.28% in BSA



**Figure 3:** Effect of eucalyptus oil + *Glycine max* extract on protein denaturation (fresh egg albumin)



**Figure 4:** Effect of eucalyptus oil + *Glycine max* extract on protein denaturation (bovine serum albumin)

*in vitro* assay. While methotrexate at concentration 100 µg/ml showed less effect compared to EO + G-max extract at a concentration (1000 µg/ml), i.e., 97.75% in egg and 93.01% in BSA *in vitro* assay, as shown in Figures 3 and 4. The maximum activity is exhibited by the herbal extract at a concentration of 1000 µg/ml. However, taking in-consideration the side effects as well as costs of allopathic drugs, natural remedies from such herbs would be more preferable option for better results.

From the results of the present study, it can be stated that the combined extract of EO and G-max is capable of controlling the production of autoantigen and inhibiting the protein denaturation in inflammation study. Hence, *E. globulus* and G-max combination can be successfully used in the management of inflammation. The effect may be due to the synergistic effect rather than a single constituent.

## Conclusion

The *in vitro* study by the inhibition of protein denaturation method conducted on the Ayurveda herbal plant combination concluded that the G-max and EO exhibited significant anti-inflammatory activity and hence can be used effectively in the management of inflammatory disorders.

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## Authors' Contributions

P.S. wrote the first draft of the manuscript. P.J. first revised the manuscript. Other authors further improved the manuscript.

## References

- Bouzd D, Mihoub ZM. Evaluation of *in vitro* anti-inflammatory activity of *Helichrysum italicum* (Roth) G. Don essential oil. *Der Pharm Lett* 2016;8:41-4.
- Leelaprakash G, Mohandass S. *In vitro* anti-inflammatory activity of methanol extract of *enicostemma axillare*. *Int J Drug Dev Res* 2011;3:189-96.
- Ferrero-Millani L, Nelsen OH, Anderson PS, Girardin SE. Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1 beta generation. *Clin Exp Immunol* 2007;147:227-35.
- Verma S. Medicinal plants with anti-inflammatory activity. *J Phytopharmacol* 2016;5:157-9.
- Artis D, Spits H. The biology of innate lymphoid cells. *Nature* 2015;517:293-301.
- Isailovic N, Daigo K, Mantovani A, Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. *J Autoimmun* 2015;60:1-11.
- Pedraza-Ava G, Perez-Martinez L, Valdez-Hernandez L, Meza-Sosa KF, Ando-Kuri M. Negative regulation of the inflammasome: Keeping inflammation under control. *Immunol Rev* 2015;265:231-57.
- Freund A, Orjalo AV, Desprez Y, Campisi J. Inflammatory networks during cellular senescence: Causes and consequences. *Trends Mol Med* 2010;16:238-46.
- Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its

- potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014;69:S4-9.
10. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein-a research. *Asian Pac J Trop Biomed* 2012;2:S178-80.
  11. Heendeniya SN, Ratnasooriya WD, Pathirana RN. *In vitro* investigation of anti-inflammatory activity and evaluation of phytochemical profile of *Syzygium caryophyllatum*. *J Pharmacogn Phytochem* 2018;7:1759-63.
  12. Sonker P, Verma S, Gupta P. To study the pharmacological effect and beneficial effect of *Eucalyptus globulus* in different types of diseases-a research. *Int J Res Pharmacol Pharmacother* 2017;6:81-3.
  13. Mei-Lin T, Chih-Chien L, Wei-Chao-Hsun Y. Antimicrobial and anti-inflammatory activities of essential oil from five herbs-a review. *Biosci Biotechnol Biochem* 1977;75:1977-83.
  14. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ. Analgesic and anti-inflammatory effects of *Essential oils of Eucalyptus*. *J Ethnopharmacol* 2003;89:277-83.
  15. Singh HP, Mittal S, Kaur S, Batish DR, Kohli RK. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*. *J Agric Food Chem* 2009;57:6962-6.
  16. Juergens UR, Stober M, Vetter H. Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1.8-cineole) in human blood monocytes *in vitro*. *Eur J Med Res* 1998;3:508-10.
  17. Muhammad KW, Naveed A, Rehan M, Jamshaid M, Muhammad H, Khan S, *et al.* Dermatological and cosmeceutical benefits of *Glycine max* (Soybean) and its active components. *Acta Pol Pharm* 2015;72:3-11.
  18. Gandhi A. Quality of soybean and its food products. *Int Food Res J* 2009;16:11-9.
  19. Mark M. Soy and health update: Evaluation of the clinical and epidemiologic literature. *Nutrients* 2016;8:754-6.
  20. Yoon EK, Hyun KK, Song C, Kim YH, Lee SH. Soybean glyceollins mitigate inducible nitric oxide synthase and cyclooxygenase-2 expression levels via suppression of the NF-KB signaling pathway in raw 264.7 cells. *J Nutr Biochem* 2012;29:1053-61.
  21. Shelke PS, Jagtap PN, Tanpure PR. Evaluation of *in vitro* anti-arthritis activity of *Boswellia serrata* and aloe barbadensis against the denaturation of protein. *Int J Sci Res* 2020;9:1-2.
  22. Pavithra TK, Smitha KP, Kulasekar KS, Kumar BS. Evaluation of *in vitro* anti-arthritis activity of *Vitex negundo* against the denaturation of protein. *Int J Curr Microbiol Appl Sci* 2015;4:87-90.
  23. Grant NH, Album HE, Kryzanasuskas C. Stabilization of serum albumin by antiinflammatory drugs. *Biochem Pharmacol* 1970;19:715-22.
  24. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol* 1968;20:169-73.
  25. Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. *J Exp Med* 1962;115:597-608.
  26. Williams LA, Connar AO, Latore L, Dennis O, Ringer S, Whittaker JA, *et al.* The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J* 2008;57:327-31.