



**DETERMINATION OF IL-10 EXPRESSION IN
PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs)
TREATED WITH AQUEOUS EXTRACT OF *GYNURA
PROCUMBENS***

By

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ABSTRACT

Gynura procumbens (*G. procumbens*) is a traditional medicinal herb that commonly used in South-East Asia as food consumption to treat inflammation, skin rashes and viral infection. Interleukin-10 (IL-10) is an anti-inflammatory cytokine act to activate macrophages and B cells, inhibit mast cells and T-helper 1 (Th1) cells, and stimulate T-helper 2 (Th2) cells. This cytokine activated from monocytes, macrophages, and different T-cell subsets which can be mainly found in peripheral blood mononuclear cells (PBMCs). Our aim was to determine the expression of IL-10 in PBMCs treated with *G. Procumbens* aqueous extraction. Peripheral Blood Mononuclear Cells (PBMCs) isolated from whole blood by using Ficoll gradient density were used to determine the activities of *G. Procumbens* on lymphocytes, monocytes and macrophages function to induce the expression of inflammatory cytokines, IL-10 with different concentrations of *G. Procumbens* aqueous extraction (50, 200 and 800 µg/ml). Viability of PBMCs was observed and calculated after staining with Trypan blue and Wright stain. A specific Toll-like receptor (TLR) 4 inhibitor, CLI095 was used to determine whether or not *G. Procumbens* exerts its effects through TLR4. An antagonist of lipopolysaccharides (LPS), polymyxin B, was used to evaluate whether endotoxin properties in *G. Procumbens* contributed to its immunomodulatory activity. Cell culture supernatants were analysed by using Luminex® Human Screening Magnetic Assay. *G. procumbens* exhibited not significantly potent immunomodulatory activity of IL-10 by PBMCs. The expression of IL-10 were increased upon exposure to different concentrations of *G. procumbens* by co-treatment with CLI-095 (TLR4 inhibitor) but partially decreased by polymyxin B (LPS-neutralizer). One-way ANOVA of those treatment groups compared to control group give $p > 0.05$ followed by post-hoc multiple comparison Dunnett's test. We demonstrate impotent immunomodulatory activity of *G. procumbens* towards the expression of IL-10 by PBMCs. We believe that this immunomodulatory activity is due to endotoxin-like properties of *G. procumbens* via TLR4 receptor and MyD88 or Toll/IL-1R domain-containing adaptor inducing IFN- β (TRIF) - signalling pathways.

Keywords: *G. procumbens*, IL-10, inflammation, Luminex®, PBMCs

CHAPTER 1

INTRODUCTION

1.1 Introduction

Medicinal plants give a large contribution towards human health and well-being in decades that still largely unexplored despite nowadays modern technology. Medicinal plants might come from different regions with different regional names or the same name, sub-species, chemo varieties and cultivars that have been providing new remedies to humankind especially in developing country (Kaewseejan et. al, 2015; Wiesner & Knöss, 2014). These medicinal plants that contain active secondary metabolites called phytochemicals and medicinal properties such as antioxidants play an important role in treatment against cancer before chemotherapy and radiation treatment (Hasani-Ranjbar, Larijani, & Abdollahi, 2009; Kaewseejan, Puangpronitag, & Nakonriab, 2012). Natural antioxidants from these medicinal plants used as an alternative products to prevent or inhibit the risk of degenerative diseases caused by oxidative damage (Kaewseejan & Siriamornpun, 2015). Various phytochemical medicines have been found to enhance the inflammatory pathways based on the profound effect and immunomodulation activities of certain components in the traditional plant. Thus suggest that plant-derived secondary metabolites as natural products can further develop from immunomodulators into immunotherapies (Wen, Chen, & Yang, 2012). An immunomodulator in medicinal plants usually used for treatment or control of infections, immunodeficiency and cancers can be defined as a substance or agent that can evoke immunomodulatory activities by enhancing or affecting immune cell systems to produce the desired immune response through dynamic regulation of the target immune systems (Spelman et al., 2006).

G. procumbens (Lour.) Merr. Compositae is an annual evergreen shrub with a fleshy stem and purple tint. This medicinal plant also called as ‘Sambung nyawa’ ‘Akar sebiak’ or “Leaves of the Gods” (Adnan & Othman, 2012) , and commonly used in South-East Asia, particularly in the Philippines, Java and Peninsular Malaysia (S.-Z. Hoe, Lee, Mok, Kamaruddin, & Lam, 2011). It has been