DETERMINATION OF INTERFERON-GAMMA IN PERIPHERAL BLOOD MONONUCLEAR CELLS TREATED WITH AQUEOUS EXTRACT OF *Gynura procumbens*

By

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ABSTRACT

Determination of Interferon-gamma in Peripheral Blood Mononuclear Cells treated with Aqueous Extract of *Gynura procumbens*

Gynura procumbens (Lour.), locally known as "sambung nyawa" is annual evergreen shrub that can be found in Southeast Asia. *G.procumbens* is used to treat many ailments such as inflammation. *G.procumbens*' anti-inflammatory and immunomodulatory activity may be utilized for treatment of inflammatory disease. The immune system play crucial role in in the pathogenesis of inflammatory disorders which can be treated using drug that have anti-inflammatory and immunostimulant properties. Interferon-gamma (IFN-ɣ) is type II interferon family, secreted by activated T cell and Natural Killer (NK) cells plays role in macrophage activation, inflammation, T helper (Th 1 cell responses) and immunoediting. Cytokine such as Interferon-ɣ is detected using peripheral blood mononuclear cells (PBMC) because PBMC contain lymphocytes that consist of T cell, B cell, NK cells, monocytes and dendritic cells (Miyahira, 2012). Although *G.procumbens* has known to have anti-inflammatory properties, the expression of Interferon-gamma towards *G.procumbens* have yet to be fully discovered. Therefore, we would like to determine the expression of Interferon-ɣ in PBMC treated with aqueous extract of *G.procumbens* using Luminex Human Magnetic Assay. In the present study, we first demonstrated the phytochemical properties of *G.procumbens.* Then, 12 ml of whole blood is separated using Ficoll density gradient centrifugation to obtain Peripheral blood mononuclear cells (PBMC). PBMC is treated with *G.procumbens* with addition of CLI 095 and Polymyxin B as inhibitor. The expression of Interferon-ɣ is measured using Luminex assay. The results show that the expression of Interferon-ɣ in PBMC treated with aqueous extract of G.procumbens showed extremely low value in three different concentration and test group. The results for all groups showed extremely low value (<49.259 Þg/ml) of expression towards Interferon-ɣ where statistically analysis were not necessary. In conclusion, we demonstrate the Peripheral Blood Mononuclear Cells (PBMC) treated with aqueous extract of *G.procmbens* do not possess the expression of Interferon-gamma (IFN-ɣ) via TLR 4 receptors and MyD88 pathway.

Keyword: *Gynura procumbens,* Interferon-gamma, Peripheral Blood Mononuclear Cells (PBMC), Toll-like receptor 4(TLR4), Immunomodulatory.

CHAPTER ONE
INTRODUCTION

1.1 Background

Medicinal plants are now attained popularity in developed countries as the remedies are thought to be innocuous. The remedies of the plant is natural and commonly used by the locals (Rosidah et al., 2009). In Southeast Asia, herbal plant are commonly used as an alternative choice to treat certain diseases. One of the plant that is commonly used as herbal remedies is *Gynura Procumbens*. *Gynura Procumbens*, known by the locals as 'sambung nyawa', is an annual evergreen shrub with a plump stem and purple tone. This plant is mostly found in Borneo, Java, and Peninsular Malaysia. *G.Procumbens* leaf is known as non-toxic and it has been used to treat many diseases such as diabetes, kidney disease, hypertension and cancer (Rosidah et. al, 2009). The plant has the highest total of flavonoid contents and exhibits the highest antioxidative content (Kaewseejan & Siriamornpun, 2015). A few research have been conducted regarding the pharmacological activities of *G.Procumbens*. Iskandar et. Al (2002) stated that the aerial part of *G.Procumbens* showed inflammatory properties and Abrika et al. (2013) stated that *G.Procumbens* can be used as an alternate medicine against increasing of blood pressure.

Interferon-gamma (IFN- ɣ), an essential facilitator of immunity and inflammation that develops the Janus Kinase (JAK) –Signal Transducer Activation of Trancription (STAT) signalling pathway to stimulate STAT1 transcription factor. IFN- ɣ signals mainly through the JAK-STAT pathway to attain transcriptional activation of IFN- ɣ inducible genes. IFN- ɣ stimulates the promotion of innate immune responses by activation of macrophages. The macrophage activation is straight effector gene initiation via STAT 1, toll-like receptor (TLR) ligands, tumour necrosis factor (TNF) and type I IFN. IFN- ɣ primes macrophages for