INTISARI

Makrofag adalah salah satu komponen penting dari sistem imun bawaan yang berperan dalam mempertahankan keseimbangan sistem imun melalui produksi mediator inflamasi seperti nitrit oksida (NO) dan sitokin interleukin-1β (IL-1β). NO berperan dalam proses fagositosis patogen dan IL-1β berperan dalam mengaktifkan berbagai sel imun lainnya untuk memberikan pertahanan terhadap infeksi patogen. Sambiloto [*Andrographis paniculata* (Burm. F) Nees] diketahui memiliki aktivitas imunomodulator baik sebagai imunostimulan maupun imunosupresan**.** Efek farmakologis yang saling bertolak belakang tersebut diduga berasal dari aktivitas senyawa yang berbeda. Sambiloto mengandung senyawa utama andrografolid, flavonoid, iridoid dan asam organik. Pemisahan senyawa dalam ekstrak dapat dilakukan dengan menggunakan metode fraksinasi. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh ekstrak air herba sambiloto dan fraksinya terhadap respon imun melalui produksi NO dan sitokin IL-1β pada sel RAW 264.7.

Ekstrak air herba sambiloto diperoleh dengan mengekstraksi simplisia herba sambiloto menggunakan metode infusa. Ekstrak air difraksinasi dengan metode *liquid-liquid continuous extraction* (LLCE) dan dilanjutkan dengan metode *Vacum Liquid Chromatography* (VLC) untuk mendapatkan fraksi A, B, C dan D. Terhadap Ekstrak air dan fraksi A, B, C, D dilakukan karakterisasi senyawa flavonoid dan andrografolid secara kualitatif menggunakan metode *high-performance thin-layer chromatography* (HPTLC), sedangkan secara kuantitatif dengan metode sepektrofotometer UV-Vis dan HPTLC-densitometri. Untuk menentukan konsentrasi ekstrak air dan fraksi A, B, C, D yang tidak toksik terhadap viabilitas sel RAW 264.7 dilakukan uji MTT *assay.* Aktivitas imunomodulator diketahui dengan melihat pengaruhnya terhadap produksi nitrit oksida (NO) dan sitokin IL-1β pada sel RAW 264.7. Uji produksi NO dilakukan penambahan reagen *Griess* dan hasilnya dianalisis menggunakan ELISA *reader*. Produksi sitokin IL-1β dilakukan dengan metode ELISA. Data kuantitatif yang diperoleh kemudian dianalisis secara statistik dengan taraf kepercayaan 95% menggunakan uji *one-way* ANOVA.

Hasil uji produksi NO dan sitokin IL-1β menunjukkan bahwa ekstrak air, fraksi A dan fraksi D dapat meningkatkan produksi NO dan sitokin IL-1β secara signifikan dibandingkan dengan kontrol sel (p<0.05), sedangkan fraksi B dan C tidak dapat meningkatkan produksi IL-1β secara signifikan dibandingkan kontrol sel (p<0.05). Kandungan senyawa aktif yaitu flavonoid pada ekstrak air herba sambiloto dan fraksinya dapat mempengaruhi produksi NO dan sitokin IL-1β pada sel RAW 264.7. Sebanyak 76.8% kadar flavonoid total ekstrak air herba sambiloto dan fraksinya mempengaruhi produksi NO dan sebanyak 58.2% mempengaruhi produksi sitokin IL-1β.

Kata kunci: imunomodulator, *Andrographis paniculata* (Burm. F) Nees, flavonoid, nitrit oksida, IL-1β.

ABSTRACT

Macrophages are one of the essential components of the innate immune system that play a role in maintaining the immune system's balance through the production of inflammatory mediators such as nitric oxide (NO) and the cytokine interleukin-1β (IL-1β). NO plays a role in the phagocytosis of pathogens, and IL-1β plays a role in activating various other immune cells to defend against pathogenic infections. Sambiloto [*Andrographis paniculata* (Burm. F) Nees] is known to have immunomodulatory activity as both immunostimulant and immunosuppressant. These contradictory pharmacological effects are thought to stem from the activity of different compounds. Sambiloto contains the main compounds andrographolide, flavonoids, iridoids, and organic acids. The separation of compounds in the extract can be done by using the fractionation method. This study aimed to determine the effect of the aqueous extract of sambiloto herb and its fraction on the immune response through the production of NO and IL-1β cytokines in RAW 264.7 cells.

The water extract of sambiloto herb was obtained by extracting the simplicia of sambiloto herb using the infusion method. The aqueous extract was fractionated using the liquid-liquid continuous extraction (LLCE) method and followed by the Vacum Liquid Chromatography (VLC) method to obtain fractions A, B, C and D. The aqueous extract and fractions A, B, C, D were characterized for flavonoid compounds. and andrographolide qualitatively using high-performance thin-layer chromatography (HPTLC), while quantitatively using UV-Vis spectrophotometer and HPTLC-densitometry methods. To determine the concentration of aqueous extracts and fractions A, B, C, D which were not toxic to the cell viability of RAW 264.7, the MTT assay was performed. The immunomodulatory activity was determined by looking at its effect on the production of nitric oxide (NO) and IL-1β cytokines in RAW 264.7 cells. The NO production test was carried out by adding Griess reagent and the results were analyzed using an ELISA reader. The production of IL-1β cytokines was carried out by the ELISA method. The quantitative data obtained were then analyzed statistically with a 95% confidence level using the one-way ANOVA test.

The test results for the production of NO and IL-1β cytokines showed that aqueous extract, fraction A and fraction D could significantly increase the production of NO and IL-1β cytokines compared to control cells ($p<0.05$). In contrast, fractions B and C could not increase NO and IL-1β production significantly compared to control cells ($p<0.05$). The content of active compounds, namely flavonoids in the aqueous extract of sambiloto herbs and their fractions, can affect the production of NO and IL-1β cytokines in RAW 264.7 cells. As much as 76.8% of the total flavonoid content of sambiloto herb water extract and its fraction affected NO production, and as much as 58.2% affected IL-1β cytokine production.

Keywords: immunomodulatory, *Andrographis paniculata* (Burm. F) Nees, flavonoids, nitric oxide, IL-1β.