ABSTRACT

Comparation of Agar Diffusion and Microdilution Methods For Growth Inhibitory Activity Test Of Aqueous Extract Of Rosella (*Hibiscus sabdariffa* L.) Calyx Against *Escherichia coli* ATCC 8739

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Aqueous extracts of roselle calyx has antibacterial activities against gram positive and negative bacteria including *Escherichia coli*. Some methods such as agar diffusion and microdilution were used to antibacterial activity test of roselle. However the recommendation of better methods among these methods has not been previosusly reported. Thus, this study attempts to compare the agar diffusion and microdillution method for inhibitory activity test of aqueous extracts of roselle calyx against *Escherichia coli* ATCC 8739 based on validation parameters among other, Limit of Detection (LOD), linearity, precision, and selectivity. It was found that the MIC or LOD of agar diffusion and microdillution methods with IC_{50} were 1% and 1.06% respectively. Correlation coefficient *r* and *Vxo* from linear equation between log concentration and inhibition zone of the agar diffusion method was 0.992 and 2.60% respectively with repeatability and intermediate precision of 1.49% and 2.86%. Correlation coefficient *r* and *Vxo* from linear equation between log concentration and death of cell (%) of the microdillution method 0.9585 and 13.17% respectively with repeatability and intermediate precision of 4.23% dan 3.27%. The obtained correlation factor of both methods indicated linearity. Agar diffusion and microdillution also has a good selectivity because they can give different response to the almost similiar samples in the same concentration. The result validat concluded that the agar diffusion method was valid and suitable for antibacterial activity test of aqueous extracts of roselle calyx against *Escherichia coli* ATCC 8739 and there was nodifferences between these methods, but microdillution method can not used of aqueous extracts of roselle calyx, because the aqueous extracts of roselle calyx can affect the absorbance of the result. So Agar diffusion method has the advantage of being more sensitive because agar diffusion more meet validation parameters.

Keywords: method validation, rosella, *E.coli* agar diffusion, microdilution