

## SUMMARY

Red amaranth (*Amaranthus tricolor* L.) is widely cultivated in Indonesia and is known to contain various secondary metabolites, including steroids. Previous studies on another species, *Amaranthus spinosus*, have reported that the n-hexane fraction contains several non-polar secondary metabolites, including sterols. Sterols are a subgroup of steroids characterized by the presence of a hydroxyl (-OH) group attached to the steroid nucleus and are commonly found in plants as phytosterols. Along with the increasing prevalence of bacterial resistance to synthetic antibiotics, the demand for natural bioactive compounds with antibacterial potential has grown considerably. Although the antibacterial activity of n-hexane fractions from various plants has been extensively investigated, no study has specifically reported the antibacterial activity of the n-hexane fraction of red amaranth. Therefore, this study aimed to isolate sterol compounds from the n-hexane fraction of red amaranth leaves and evaluate their antibacterial activity.

The research procedures included plant identification, sample preparation, phytochemical screening, extraction, and fractionation to obtain the n-hexane fraction, which was subsequently subjected to antibacterial testing. Antibacterial activity was evaluated against test bacteria using the agar well diffusion method to determine the inhibitory potential of compounds present in the n-hexane fraction. The n-hexane fraction was further separated using gravity column chromatography, thin-layer chromatography (TLC), and preparative TLC to isolate sterol compounds. The resulting isolate was subjected to purity assessment by TLC and characterized using Liquid Chromatography–Mass Spectrometry (LC–MS).

The results of phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids in red amaranth leaves. Maceration of the leaves yielded an ethanolic extract with a percentage yield of 6.97%, while the n-hexane fraction showed a yield of 4.96%. Antibacterial assays demonstrated that the n-hexane fraction exhibited inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*, with the largest inhibition zones observed after 12 h of incubation, measuring 12.00 mm and 10.50 mm, respectively, at the highest concentration tested. Based on TLC analysis, positive coloration with the Liebermann–Burchard reagent, and LC–MS characterization, the isolate was presumed to contain a steroidal compound belonging to the phytosterol class, showing structural similarity to spinasterol.