

ABSTRACT

Therapeutic potential of *Andrographolide*, a bioactive compound in *Andrographis paniculata* extract that has anti-inflammatory, antibacterial, and antitumor activities, has been used as a traditional medicine in Asian countries. *Andrographolide* is also reported to have antiviral activity including potential treatment against SARS-Cov-2 virus infection, causing an increase in demand for Sambiloto-based products. This study aimed to quantify the *Andrographolide* content in Sambiloto herbal preparations using UV-Vis Spectrophotometry, HPLC, and ¹H-NMR, with dibenzyl ether as an internal standard. The UV-Vis analysis showed no significant difference in average values of *Andrographolide* content: 2.54% and 2.39% using external and internal standards, respectively. The use of UV-Vis spectrophotometer has limitations due to the presence of other components that absorb at the same wavelength and can alter the test findings, especially for complex compounds. HPLC analysis, under optimal conditions (Acetonitrile: 0.1% o-phosphate, 40:60), revealed a content of 0.1925%. UV and HPLC typically calls for pricey pure reference materials to ascertain the component composition in the extract. Using dibenzyl ether, qHNMR analysis revealed a content of 0.231%, which is in line with the mass percentage determined by HPLC. Dibenzyl ether showed effectiveness as a novel internal standard in qHNMR analysis due to its stability, inertness to the target compound, sharp singlet proton signal, and lack of overlap with the target compound signal, it also provides a more affordable option than pricey pure standards. This research increases convenience and contributes to consumers, the development of science, and the pharmaceutical industry, in the standardization of Sambiloto-based herbal products.

Keywords: qHNMR, Dibenzyl ether, Internal standard, Herbal preparations, Quantification