Abstract: *Vitex negundo* has been known since ancient times as a medicinal plant. The objective of this study is to investigate the effect of methanol and ethanol extracts, and ethyl acetate, chloroform and aqueous fractions of *Vitex negundo* using an *in vitro* model to test glucose diffusion and to determine the phytochemical profile of the extracts and fractions using 13C nuclear magnetic resonance (NMR) spectroscopy. The chloroform fractions, ethyl acetate-EtOH and ethyl acetate-MeOH gave the highest inhibitory effect on both the diffusion activities in vitro. Retardation of glucose diffusion suggests that *V. negundo* has the potential to lower postprandial glucose. Correlation analysis of the 13C NMR profile with retardation activity suggests that compounds containing glycosidic residues may be responsible for the glucose retardation activity. This is the first example where activity has been correlated with specific structural features of compounds from a crude extract using 13C NMR chemical shifts to assist in the identification of active compounds.

Keywords: *Vitex negundo*, *in vitro*, glucose diffusion retardation index, antidiabetic, 13C NMR