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Three assay methods for screening of HIV-1 protease inhibitors, spectrophotometry, high performance liquid chromatography (HPLC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), were compared for the selection of the most appropriate method for screening of HIV-1 protease inhibitors of some medicinal plants and chemical compounds. Spectrophotometry and HPLC were used to detect the product, Phe(NO_2)-Glu-Ala-Nle-Ser-NH_2, which was cleaved from substrate, His-Lys-Ala-Arg-Val-Leu-Phe(NO_2)-Glu-Ala-Nle-Ser-NH_2, by recombinant HIV-1 protease enzyme. SDS-PAGE was used to detect the intensity of the substrate band which was decreased by enzymatic reaction. Water (or dimethyl sulfoxide (DMSO)) and pepstatin A were used as negative and positive control, respectively. The results showed that spectrophotometric method was the most appropriate method for screening of HIV-1 protease inhibitors. Water extracts from six medicinal plants and ethanol extracts of nine medicinal plants with concentration of 5 μg/μl, and eight chemical compounds (No.1-8) with concentration of 20 μg/ ml were tested for HIV-1 protease inhibitory effect. For water extracts, the results showed that *Momordica charantia* Linn. exhibited inhibitory activity on HIV-1 protease with 78.95%. *Ipomoea crassicaulis* Roxb. and *Vitex negundo* Linn. exhibited inhibitory activity on HIV-1 protease with 57.89%. *Canna orientalis* Rosc. and *Vitex trifolia* Linn. exhibited inhibitory activity on HIV-1 protease with 100%. *Justicia vulgaris* Nees. did not inhibit HIV-1 protease *in vitro*. For ethanol extracts, the results showed that *Justicia valida* Ridl. var. *grandulosa* Fisch., *Andrographis paniculata* (Burm.) Wall. ex. Nees., *Vitex trifolia* Linn., and. *Vitex negundo* Linn. exhibited inhibitory activity on HIV-1 protease with 100%. *Clausena excavata* Burm. f., *Ipomoea crassicaulis* Roxb., *Canna orientalis* Rosc., and *Argyreia nervosa* (Burm. f.) Boj. exhibited inhibitory activity on HIV-1 protease with 78.95, 68.42, 56.52, and 4.35% respectively. *Justicia vulgaris* Nees. did not inhibit HIV-1 protease *in vitro*. For chemical compounds, compounds 1, 2, 3, 4, 6 and 8 exhibited inhibitory activity on HIV-1 protease with 28.57, 33.33, 88.10 79.52, 57.14, and 57.14%. Compounds 5 and 7 did not inhibit HIV-1 protease *in vitro*.