Abstract

Andrographis paniculata is a well known plant in Asia. It is used in traditional medicine for antimalarial, antibacterial, antiviral, anticancer, antipyretic, analgesic, anti-inflammatory, and sexual stimulationand increase sperm production. The highest active constituent in *A. paniculata* is andrographolide (SS1), 0.6%. Andrographolide (SS1) is an *ent*-labdane diterpene, its 13-16 carbon forming a γ-lactone ring. There are 3 free hydroxyls at positions 3, 14 and 19. In this experiment, esterifications of these three OHs with long and short chains fatty acids were carried out. In this study, 11 analogues of andrographolide were synthesized and tested for biological activities; they are 1) 3,19-Dipalmitoylandrographolide (SS40), 2) 14-deoxy-11,12-didehydro-3,19-dipalmitoylandrographolide (SS17), 3) 3,19-isopropylideneandrographolide (SS3), 4) 14-acetylandrographolide (SS19) 5) 3,14,19-triacetylandrographolide (SS20B), 6) 3,14,19-tripalmitoylandrographolide (SS39), 7) 14-monoacetyl-3,19-isopropylideneandrographolide (SS34A), 8) 14-deoxy-11,12-didehydro-3,19-isopropyrideneandrographolide (SS27RT).

All compounds lost the bitter taste as found in andrographolide. In the studying of the biological activity analogues were compared with the naturally occurring compounds which are andrographolode (SS1), 14-deoxy-11,12-didehydroandrographolide (SS2), 14-deoxyandrographolide (AND2). Acetoyl and palmitoyl analogues were used to test for antibacterial, anti-HSV-1, and reversed transcriptase inhibition for anti-HIV activity, antipyretic, analgesic, anti-inflammatory and sexual stimulation. It was found that the acetoyl analogues are active against *Staphyllococcus aureus, Enterococcus faecalis and Bacillus subtilis.* It was found that 14-acetylandrographolide, the most active analogue, exerted the effect on the cell wall of *B. subtilis*

which is the most subceptible bacteria. For anti-HSV-1, all acetoyl analogues were found to be active at the pre-viral infection stage. Whereas the most active post infection stage is SS3 which exerts the activity at the early stage of DNA synthesis. Meanwhile SS2 gave the highest activity on reverse transcriptase against the HIV (10 μM, 84%) and followed by SS39 (72.44%, 6.46μM) and SS1 (67%, 16 μM).

Since SS3 is very effective and showed absolute activity in antireplication of HSV-1, the partition coefficient of the compound was compared to the natural occurring compounds, SS1, SS2, AND. An analytical HPLC method simultaneous determination of SS1, SS2, AND and SS3 was establish to determine the partition coefficients of the four compounds. The quantitation limits were 4.72 to 5.21μg/ml. The accuracy were 98.7±5.3996.7, 100.3±6.77, 96.7±5.25, 104.7±2.75% recovery, respectively. The intraday variations were 0.06,0.08,0.06 and 0.07%RSD, the interday variations were 0.57, 3.01, 1.76, and 0.74%RSD. The linearity ranges were 18.6-223.5, 13.0-157.5, 16.0-192.0 and 12.5-150.0 μg/ml. with the regression of 1.0000, 0.9995, 0.9998, and 0.9999, respectively. Partition coefficients were 43±3.96, 374.9±4.31,348.1±9.04 and 1017.3±7.5, respectively.

For pharmacological activity, SS1, SS2, SS19, SS3 and SS17 were intraperitoneally tested for their analgesic, antipyretic, anti-inflammatory and acute toxicity effects in animal models. Analgesic effects was tested in mice using hot plate and writhing tests to distinguish the central and peripheral effects, respectively. The results showed that, at 4 mg/kg, all tested substances have significant analgesic effects, and the highest potency was seen with SS3, SS19 and SS17. Increasing the dose of SS 3 and SS17 to 8 mg/kg did not increase the analgesic effect. In the

writhing test, SS3 and SS17, but not SS1, showed significant results. Meanwhile, in a baker's yeast-induced fever model, SS3 and SS17 significantly reduced rats' rectal temperature ($p<0.05$). In a carrageenan-induced inflammation model, SS1, SS3 and SS17 significantly reduced rats' paw volume. Doses of SS3 and SS17 up to 100 mg/kg did not show any serious toxic effects. From this study, SS3 and SS17 are the most interesting derivatives, showing much greater potency than their parent compounds.

For the other pharmacological activities, SS3 and SS17 showed no activity on aggressiveness, anxiolytic effect and antidepressive effect.

However, intraperitoneally injection of at the dose of 50mg/kg of SS17 increase the mounting frequency in days 1-4 but not equivalent to sildenafil, the positive drug control. However, 50mg/kg of SS1 when orally administered for 2, 4, 6 and 8 weeks to mice and rats, increased the blood testosterone level significantly in week 4 and decreased in weeks 6 and 8. There was no change in sperm movement. In endothelium-intact rat aortic strips, norepineprine-induced contraction was reduced by preincubation with andrographolide.

Keywords: Andrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, 3,19-Dipalmitoylandrographolide, 14-deoxy-11,12-didehydro-3,19-dipalmitoylandrographolide, 3,19-isopropylideneandrographolide, 14-acetylandrographolide, 3,14,19-triacetylandrographolide, 3,14,19-tripalmitoylandrographolide, 14-monoacetyl-3,19-isopropylideneandrographolide, 14-deoxy-11,12-didehydro-3,19-isopropyrideneandrographolide, anti-HSV-1, anti-replication of HSV-1, HSV-1 pre-infection, reverse transcriptase inhibition, HIV, antibacterial, analgesic, antipyretic, anti-inflammatory, male sexual function, blood testosterone level