URN etd-0407109-183726-2148
Author Yu-Min Huang
Author's Email Address No Public.
Statistics This thesis had been viewed 49 times. Download 0 times.
Department
Year 2004
Semester 2
Degree Master
Type of Document Master's Thesis
Language zh-TW.Big5 Chinese
Title Study of the Whitening Function and Anti- oxidation Properties and Promoting Whitening Function of Wu-Bai Chinese Herbal
Date of Defense 0000-00-00
Page Count 130

Keyword
Whitening
Wu-Bai Chinese Herbal
Anti-oxidation
Tyrosinase
Soxhlet extraction
wet-mill

Abstract
In this study, we conducted in vitro test in which the abilities of whitening and anti-oxidation of Wu-Bai Chinese herbs composite prepared by wet-mill method and soxhlet-extraction method were defined. Wu-Bai Chinese herbs composite is found to have whitening function by Chi-Mai Medical Center. The purpose of wet-mill is to reduce the particle size of this Chinese herb and increase its surface area. As in soxhlet-extraction method, the Wu-Bai herb composite were extracted with methanol as well as 95 %, 50 % and 30 % aqueous solutions of Ethanol to obtain a highly concentrated effective ingredients. Both the wet mill-ed sample and the extracted ingredients were then formulated as gel form or lotion and tested for its whitening function in human skin.

The particle size of Wu-Bai herb composite was reduced from 75 μm to 600 nm by 24 hours wet-mill procedure. From the test in vitro, we found that the abilities of tyrosinase inhibition and anti-oxidation of Wu-Bai Chinese herb increase as its concentration increases. And milled Wu-Bai Chinese herb powder is more effective than original Wu-Bai herb powder due to its smaller particle size, which should provide larger specific surface area and smaller resistance of mass transfer. As for the extract of Soxhlet extraction, since solvent extraction removes most of impurities, the active ingredients per gram of extract is increased, therefore the results of tests were better than that of original powder and milled powder.

For concentration of 10 mg/ml, every gram of Wu-Bai herb gave 33.46±0.05 mg phenolic compounds while extracted with 50 % aqueous solution of ethanol, which rank the highest in yield. The rest of yield was as following: 95 % aqueous solution of ethanol > methanol > 30 % aqueous solution of ethanol. When concentration reaches 60 mg/mL, only the extract of 50 % aqueous solution of ethanol exhibits more than 50 % of inhibition in tyrosinase activity and the IC50 value is 55.3 mg/mL; In the inhibition test of dopachrome autoxidation, the extract from aqueous solution of 50 % ethanol gave the best performance: when concentration is 70 mg/mL, the inhibition percentage is 75.2 %; The best lag time in total anti-oxidation activity test is 135 seconds, which was given by extract from aqueous solution of 30 % ethanol and no discoloration was observed either. However, extract from 50% aqueous solution of ethanol ranked the best in the ability test of total reduction where IC50 is 3.99 mg/mL. This result may suggest the difference in active ingredients. Wu-Bai

Chinese herbs composite is effective in terms of DPPH free radical scavenging test, hydrogen peroxide scavenge test and ferrous iron chelated test. Among all samples, the extract of 50 % aqueous solution of ethanol showed the best result. Results from the whitening test performed by applying above-mentioned formulations on the arms of 10 human subjects for 15 days indicated the trend of reduction in the melanin index. Again, the extract from 50 % aqueous solution of ethanol is the most effective one. Besides, formulated in lotion form showed at least 6% better result than that of gel form.

Furthermore, extraction were performed for each constituents of the Wu-Bai Chinese herbs composite, Angelicaedahuricae radix, Aconiticoreani tuber, Proia cocos, Ampelopsis radix and Herbal A, with 50 % aqueous solution of ethanol and the whitening and antioxidation test were conducted respectively. All samples showed very similar results while the extract of Angelicaedahuricae radix was the best among all. In addition, the results also suggest that Aconiticoreani tuber and Herbal A may contribute to the discoloration in lag time of total anti-oxidation test.
Advisory Committee Liao Wei-Tong - advisor

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Date of Submission 2005-03-25