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Bioactivity-Guided Screening of Wound-Healing Active Constituents from American Cockroach (*Periplaneta americana*)

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Abstract: Ethanol extract (EE) from *Periplaneta americana* (PA) is the main ingredient of Kangfuxin, which is a popular traditional chinese medicine (TCM) and has long been used for the clinical treatment of burns, wounds and ulcers. We compared the wound-healing activities of three extracts of PA using cutaneous wound-healing in mice as the bioactivity model. These three extracts were EE, total polysaccharide and total protein. We also tracked bioactive fractions in the EE by organic reagent extraction, column chromatography and HPLC. Seven compounds were successfully identified from the water elution fraction of the EE of PA using UPLC-MS. Among these compounds, four compounds (**P2**, **P3**, **P4**, **P5(1)**) were first reported in PA. Some of these compounds have been previously reported to have various pharmacological activities that could contribute to the high wound-healing activity of PA.

Keywords: *Periplaneta americana; wound-healing activity; active constituents; bioactivity-guided screening*

1. Introduction

Wound healing is a complex biological process including three classic stages: inflammation, new tissue formation and remodeling [1]. Any aberrancies at each stage can lead to delayed wound healing. Many synthetic drugs and artificial skins are used for wound healing, but they are expensive and readily cause allergic reactions in sensitive people [2–4]. Thus, there is a real need for an alternative to synthetic wound-healing products. Natural products are the most reliable and successful sources of drug leads. For example, insects have been increasingly used as effective bioactive products and as they are one of the most diverse taxa of living organisms can provide a considerable resource of potential alternatives [5]. Therefore, the potential for insect constituents, such as cockroaches, to enhance natural wound healing is a particularly important research avenue.

Periplaneta americana (PA), the American cockroach, has the largest body size in the family Blattidae. It is also one of the most famous sanitation-related insects with strong vitality and successful reproduction [6]. PA is widely distributed in subtropical and tropical regions across the world [7]. Many previous PA studies have focused on the infestation of human dwellings and the strong ability to transmit pathogenic fungi [8]. However, other physiological and pharmacological studies have demonstrated that PA constituents have favorable tissue-repairing [9], antibacterial [10], antitumor [11] and immunity-enhancing activities [12]. Additionally, this insect has been used in traditional chinese medicine (TCM) as an important biomedical component for the treatment of TCM syndromes such as

blood stasis, ulcers, burns and wounds for hundreds of years [13]. Consequently, the formulations of many TCM preparations, such as Kangfuxin Liquid, Ganlong Capsule, Xinmailong Injection, and Xiaozheng Yigan Tablet, include ingredients from PA [14]. Among them, Kangfuxin is a liquid preparation that has been used to treat different skin or mucosa injuries in China for more than 40 years [15]. According to the previous component analysis on PA, the reported chemical components of the cockroach mainly include pheromones, amino acids, insect neuropeptides, adipokinetic hormones and dihydroisocoumarins [16]. However, the effective ingredients in PA that can promote the wound-healing process remain largely unknown. The purpose of this study is to investigate related active chemical components that demonstrate efficacy in wound healing. In the present study, we systematically screened the bioactive compounds or fractions with wound-healing activities in PA. The ethanol extract (EE), total polysaccharide and total protein of PA were used to compare their wound-healing activities on the basis of the cutaneous injured mice model. Then, we performed systematic separation and analysis on the EE of PA, including organic reagent extraction, macroporous resin column chromatography, High Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS).

2. Results and Discussion

2.1. Comparison of Wound-Healing Activities of Different PA Extracts

The wound-healing activities of the EE, polysaccharide (TPS) and total protein (TP) of PA are shown in Figure 1a. Compared to other PA extracts, the EE-treated wounds became dry, and scabs began to form on the first day of treatment, likely as a result of the adjacent skin cells interacting with the scab to promote wound healing [17]. The sizes of the wounds decreased significantly at the third and sixth day in the EE-treated group. Scabs of wounds treated with EE started to drop off on the ninth day, and the granulation tissue below the wound grew swiftly and showed a pinkish color. However, pus and blood were observed in the wounds of the TPS and TP group mice even after the sixth day. The results indicated that EE-treated wounds scabbed faster, and had smaller wound sizes and less swelling than those treated with TPS, TP and Jingwanhong (i.e., positive control—PC).

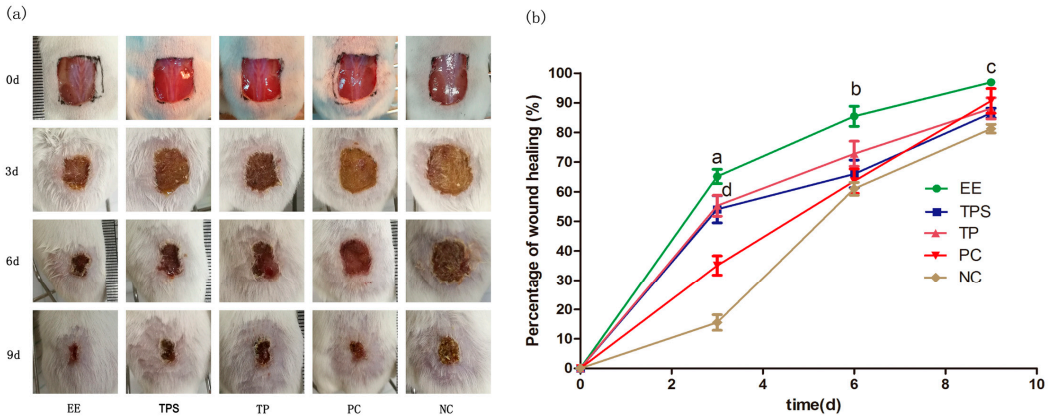

Figure 1. Wound-healing assay of mice treated with ethanol extract (EE), total polysaccharide (TPS), total protein (TP), Jingwanhong (positive control—PC) and pure water (negative control—NC) at 0, 3, 6 and 9 days. (**a**) Photographs of dorsal excisional skin wounds on different days. Day 0 pictures were taken immediately after wounding; (**b**) Data are given as mean ± standard deviation (SD) for three mice in each group. Statistical analysis of wound area used one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post hoc test. The characters indicate statistically significant differences ($p < 0.05$): a, b and c: EE compared with other four groups on days 3, 6 and 9; d: between TP/TPS and NC on day 3.