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Short communication

Preliminary screening via dose–response analysis of the antibacterial activities of six Chinese medicinal plant extracts

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ABSTRACT

This first-attempt study quantitatively provided dose–response assessment of *in vitro* antibacterial activity of typical Chinese medicinal plant extracts. The diameter of inhibition zone determined by disc diffusion method was adopted to quantify antibacterial activity of various plant extracts upon *Escherichia coli*. Our findings indicated that antibacterial activities of crude plant extracts obtained by maceration (mac.) and decoction (dec.) were significantly different. Moreover, extracts of *Phyla nodiflora* (mac.), *Coptis chinensis Franch* (mac.) and *C. chinensis Franch* (decoction) were found to be more feasible to be antibacterial agents for possible therapeutic uses. In addition, *P. nodiflora* (mac.) was very likely most cost-effective among all extracts.

1. Introduction

As the bulk of antibiotics produced in the world treats human infections and promotes growth and controls infections in the food animals, bacterial resistance to currently used antibiotics is gradually becoming a concern to treatment of infectious diseases for public health (WHO publication 2001). Apparently, alternative antibacterial agents provoked less side effects to protect humans from bacterial infection were thus in urgent demand. Due to concern in possible toxicity and side-effects of artificially-synthesized medicines, Chinese herbal medications in particular have become a revival of interest as Chinese ancestors used a variety of plant medicines to help maintain and/or stimulate human health (Cock, 2008). Extracts of many parts in plants (e.g., leaves and stems) have been shown to exert biological activity *in vitro* and *in vivo* (e.g., antimicrobial characteristics for traditional healthcare) (Kilani *et al.*, 2008; Martinez *et al.*, 1996; Nimri *et al.*, 1999). It is anticipated that plant compounds attacking target sites other than those used by antibiotics could be active against antibiotic-resistant bacterial pathogens. This study was thus systematically designed to examine antimicrobial activity characteristics of different extracts from six indigenous medicinal plants (*Abrus cantoniensis Hance* (AC), *C. chinensis Franch* (CC), *Ocimum basilicum* L. (OB), *P. nodiflora* (L.) *Greene* (PN), *Plantago major* L. (PM), *Taraxacum mongolicum* Hand. Mazz (TM)). Literature (Chiu and Chang, 2003) mentioned that these indigenous plants were popularly used in pain, fever, diarrhea, dysentery and other intestinal problems as traditional Chinese medicine. This feasibility study also provided a novel attempt (i.e., dose–response analysis) from a toxicological perspective to put forward, in quantitative terms and explanations, the rankings of *in vitro* antibacterial activity of these plant extracts.

A typical dose–response curve to quantify antibacterial activity evaluation can be described in four significant parameters: the threshold (baseline dose; EC_0), the maximum treatment concentration (top dose; EC_{100}), the concentration to provoke a 50% response (median effective dose; EC_{50}) and the slope of response curve (Chen and Chang, 2005). The EC_0 and EC_{100} are defined as the maximum concentration to have a detectable response (i.e., 0^+%) and the minimum concentration to have 100% maximum response, respectively. Within safe dosage to humans, if values of effective concentrations (e.g., EC_{50}, EC_{20}) of plant extract A are much less than those for plant extract B, A is possibly more effective than B for antibacterial activity to the indicator bacterium. Comparative analysis on antibacterial activities of myriads of plant extracts could clearly point out biologically feasible plant extracts to go forward next-step screening or trial for therapeutic applications. Here, we adopted the diameter of inhibition zone (IZ) determined by a modified Kirby–Bauer's disc diffusion method (Bauer *et al.*, 1966) to quantitatively reveal the antibacterial activities of medicinal plants upon the reporter bacterium *Escherichia coli* UVT1 (UVT1 for short). As known, Gram-positive bacteria are usually more susceptible to antibiotics than are Gram-negative

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Nomenclature

A	intercept of probit model for dose–response curves
B	slope factor (or steepness measure) of probit model for dose–response curves
C	concentration of tested extract sample (mg/mL)
EC_x	effective concentration of tested plant extract to provoke x% response (mg/mL)
$erf(x)$	error function
MIC	minimum inhibitory concentration (mg/mL)
P	response variable of dose–response curves (%)
Y	probit unit

bacteria. Here, to indicate promising plant extracts for antibacterial activity, we intentionally selected a typical Gram-negative bacterium – *E. coli* as the indicator microorganism. In addition, the measure of *in vitro* antibacterial activity response (i.e., $P\% = (D - D_0)/(D_T - D_0)$) was defined as an apparent diameter of inhibition zone (IZ) in the presence of test plant extract divided by the apparent diameter of IZ (i.e., $D_T - D_0$) in the presence of tetracycline, where D_0 and D_T denoted the diameter of the filter disc (6 mm) and IZ of tetracycline (140 μg/mL), respectively. To design all responses well distributed in (0, 100%), the measure of this antibacterial activity response is specifically defined to satisfy asymptotic behaviors as follows:

$$\text{(a) lower bound at threshold dose (Th)} : \left.\frac{D - D_0}{D_T - D_0}\right|_{C < EC_0 = Th} = 0, \quad (1)$$

$$\text{(b) upper bound at diffusion limit } (D_{max}) : \left.\frac{D - D_0}{D_T - D_0}\right|_{C > EC_{100}} = \frac{D_{max} - D_0}{D_T - D_0}. \quad (2)$$

These asymptotic behaviors postulated that (1) antibacterial activity characteristics of plant extracts followed the threshold model (mentioned afterwards), (2) the maximum diameter D value (D_{max}) for sampled plant extract would not exceed D_T. As the dose–response parameters (e.g., EC_0, EC_{50} and EC_{100}; Chen *et al.*, 2004, 2006) revealed, the order of antibacterial activity of different plant extracts could be clearly quantitatively indicated. Moreover, the consequence of plant extraction and processing significantly influenced the overall antibacterial activity of crude extracts (e.g., increasing or preserving the efficacy and bioavailability). Our findings indicated that antibacterial activities of extracts obtained by maceration (mac.) and decoction (dec.) were significantly different. Plus, antibacterial activities of PN (mac.), PN (dec.) and CC (dec.) were significantly effective to inhibit the growth of *E. coli*, indicating that these plant extracts could be economically feasible to go forward next-step development in therapeutic uses.

2. Materials and methods

2.1. Chemicals and materials

Methanol (ECHO HPLC grade), dimethyl sulfoxide (DMSO) (TEDIA HPLC/spectro) were used as solvents for extraction. To reveal antibacterial activity of plant extracts, *tetracycline* (SIGMA) and *ampicillin* (SIGMA) were used as model antibiotics for comparison. Sterile filter papers (ADVANTEC NO.5A) punched in 6 mm-diameter discs were used for antibacterial activity testing using disc diffusion method. Luria-Bertani broth (LB Broth, Miller; Difco) was used as a growth medium for cultures of indicator microorganism *E. coli* UVT1.

2.2. Plant collection and extraction

All fresh plants (i.e., PM, AC, PN, OB, CC and TM) were purchased from the local herb stores, dried at 40 °C for 2 days in a temperature-controlled oven, and then milled to be fine powders as traditional Chinese medicinal uses. All plants were identified and confirmed via Professor Dr. Ang Wu in Department of Horticulture, National I-Lan University. Plant extracts were prepared by using methanol (1.0 g powder/20 mL MeOH) via decoction (dec.) and maceration (mac.) as follows: (A) Maceration: the methanolic mixtures of plant powder were well-stirred for 24 h in completely sealed serum bottles at ambient temperature. Then, the mixtures were filtered through filter papers (ADVANTEC NO.5A) via vacuum filtration by the application of reduced pressure to obtain crude maceration extracts (CME). (B) Decoction: the methanolic mixtures collected in laboratory flask were totally refluxed for 12 h via condensation. Then, the methanol present in refluxed condensate-bearing mixtures was filtered by using filter papers (ADVANTEC NO.5A) via reduced pressure filtration through rotary evaporator at 50 °C to obtain crude decoction extracts (CDE). Samples of CME and CDE were all then dissolved in dimethyl sulfoxide (DMSO) to attain the high-concentration stock solution (HCSS) for further testing of antibacterial activity (Barbour *et al.*, 2004; Sobero *et al.*, 2007; Voravuthikunchai *et al.*, 2004).

2.3. Conceptual relations of antibacterial activity

The core perspective behind dose–response relations (Chen *et al.*, 2004, 2006; Ottoboni, 1997) is mainly to indicate the maximum treatment concentration (denoted as EC_{100}), 50% effective concentration EC_{50} (or median effective dose) and threshold concentration (i.e., maximal "no-response" concentration or EC_0) to trigger inhibition of bacterial growth for antibacterial activity. Note that EC_x indicated the effective concentration administered to the bacterial population of UVT1 to provoke x% response ($0 \le x \le 100$). A dose–response curve contained three parts: no-effect range, range of increasing effect with increasing dose (or second effect range), and maximum effect range (Ottoboni, 1997). Concentrations of crude plant extracts above and under their EC_{100} result in culture extinction and survival, respectively. To get an obvious diagram of antibacterial activity of various plant extracts, we assumed that dose–response curves followed a standard sigmoidal shape which could be described by a Log-probit model (i.e., $Y = A + B \log C$; Chen *et al.*, 2004).

The response variables in antibacterial activity can be elucidated as follows (Chen *et al.*, 2004): the diameter of IZ to UVT1 cells (i.e., $D_0 \le D_1|_{C_1} < D_2|_{C_2} < D_3|_{C_3} < \cdots < D_\infty|_{C_\infty} < D_{max} \le D_T < +\infty$ or $0 \le P_1|_{C_1} < P_2|_{C_2} < P_3|_{C_3} < \cdots < P_\infty|_{C_\infty} \cong P_T = 100$) monotonically increases with increased concentration C (i.e., $+\infty > \cdots > C_n > \cdots > C_3 > C_2 > C_1 \ge 0$). The measure of antibacterial activity response (i.e., $(D - D_0)/(D_T - D_0)$) is defined as an apparent diameter of IZ (i.e., $D - D_0$) in the presence of test plant extract divided by the diameter of IZ in the presence of tetracycline (i.e., $D_T - D_0$), where D_0 indicates the diameter of the disc (6.0 mm).

2.4. Microorganism and culture conditions

As *E. coli* is commonly found in the lower intestine of human body, we intentionally selected *E. coli* as an indicator microorganism to reveal preliminary screening of antibacterial activity of plant extracts. To standardize the culture of cells, a loopful of *E. coli* UVT1 seed taken from an isolated colony in LB-streak plate was first precultured in 50 mL LB broth for 12 h at 37 °C, 125 rpm. Then,