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Original article

Assessments of antibacterial activity, phytochemical constituents, and cytotoxicity of herbal preparations used in Thailand

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Abstract

Introduction: The present work aimed to evaluate the antibacterial activity, phytochemical constituents, and cytotoxic effect of plant based remedies which are traditionally used for the treatment of infections.
Methodology: Antibacterial activity of the ethanol extracts of plant based preparations described in Thai Pharmaceutical Textbook namely: Tri-Khan-Tha-Wart, Tri-Ke-Son-Phet, Tri-Phit-Tha-Chak, Tri-Ka-Tuk, Tri-Pha-La, Tri-Kan-La-Phit, Tri-Chin-Tha-La-Ma-Ka, Tri-San, Tri-Sa-Mo, and Tri-Ke-Son-Mat were evaluated on 20 isolates of Gram-positive and Gram-negative pathogens. Qualitative phytochemical analysis and cytotoxic effect on Vero cells of the extracts were additionally performed.
Results: The extract of Tri-Chin-Tha-La-Ma-Ka possessed the highest and most significant antibacterial activity with minimum inhibitory concentration (MIC) values of <16 μg/mL against methicillin resistant *Staphylococcus aureus* (MRSA), a biofilm-producing *Staphylococcus epidermidis*, acne lesion isolated coagulase-positive and coagulase-negative staphylococci isolates. Our preliminary phytochemical test revealed that triterpenoids and phenolics were common components found in all tested preparations. Most of the tested remedies had $IC_{50} > 50$ μg/mL on Vero cells, whereas Tri-Chin-Tha-La-Ma-Ka, Tri-Ka-Tuk, and Tri-Kan-La-Phit had IC_{50} value of 0.9, 48.8, and 28.9 μg/mL, respectively.
Conclusion: These findings suggest that *Tri-Chin-Tha-La-Ma-Ka* could be further studied as a promising antibacterial agent. Investigations on other biological activities related to its traditional applications, appropriate biomarkers, and treatment mechanisms of the preparation are required.

Keywords: Antibacterial activity; Medicinal plant preparations; Medicinal plant; Traditional Thai medicine; Herbal medicines

Introduction

Infectious diseases are the second leading cause of death worldwide and estimated to be responsible for the deaths of 15 million individuals throughout the world each year [1]. According to extensive reviews in the EU and the USA, the impact of antibiotic resistant bacterial infections was estimated to cause extra healthcare costs of at least $1.5 billion per year [2]. Approximately 25,000 patients per year die from bacterial infections, and risk of mortality of patients with antibiotic-resistant infections is two times higher than the patients without an antibiotic-resistant infection [3]. Although data from low- and middle-income countries are rare, available information reveals a similar situation in antibacterial resistance [3,4]. Major pathogens contributing to the burden of resistance in several countries are methicillin resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci (CoNS), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli* [3,5]. An increase of selective pressure from the use of antibiotics coupled with a rapid global spread of infectious diseases and a decline in the development of new antibiotics has rendered infections untreatable.

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Herbal medicines and practitioners' wisdom are recognized as important resources in healthcare in many countries, including Thailand [6]. Traditional herbal remedies in traditional Indian medicine (Ayurveda) [7] and traditional Chinese medicine [8] have become popular due to their abundant potential biological activities. Even though there are numerous reports on antimicrobial activity of crude extracts prepared from Thai medicinal plants, a limited number of studies on Thai traditional remedies have been published.

Thai traditional medical practices are divided into four categories: medical practice, pharmacy practice, traditional midwifery, and traditional massage. The scope of traditional pharmacy practice includes the study of substances used as medicine, the properties and classification of natural products for medicine, and the dispensing of herbal medicine. The traditional drugs can be classified into three groups, namely, 'Chunla Pikut' (this means 'small class' and includes preparations made from equal parts of two medicinal substances under the same local name), 'Pikut Ya' (this means 'large class' and it is made from equal parts of at least two medicinal substances), and 'Maha Pikut' (this means 'great class' and it is made from unequal parts of three to five medicinal substances) [9]. 'Pikut Tri' is the largest group of traditional formulas in 'Pikut Ya', consisting of equal parts of three medicinal substances ('Pikut' means a group of medicinal substances and 'Tri' means three). There are 33 different types of remedies described in the Thai Pharmaceutical Textbook [10]. Commonly, the preparations are used as tea, an infusion of the medicinal plants, or they are added into other traditional formulas. The selection of the 10 remedies used in this study was based on their traditional use for the treatment of infectious related ailments [11]. The aims of the paper were to evaluate (i) *in vitro* antimicrobial properties against seven Gram-positive and Gram-negative multi-drug resistant bacteria species, (ii) cytotoxicity and (iii) phytochemical constituents of ethanol extracts of selected Pikut Tri.

Materials and methods

Preparation of Thai ancient remedies and their extracts

The list of medicinal components of ten selected 'Pikut Tri' is summarized in Table 1 [9]. The tested remedies were (1) Tri-Khan-Tha-Wart, (2) Tri-Ke-Son-Phet, (3) Tri-Phit-Tha-Chak, (4) Tri-Ka-Tuk, (5) Tri-Pha-La, (6) Tri-Kan-La-Phit, (7) Tri-Chin-Tha-La-Ma-Ka, (8) Tri-San, (9) Tri-Sa-Mo, and (10) Tri-Ke-Son-Mat. Plant materials were purchased from a licensed traditional medical drug store, Triburi Orsot in Hat Yai, Songkla, Thailand. Reference specimens of the materia medica were deposited at the Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand. The materials were cleaned and dried at 60 °C overnight. According to Thai Pharmaceutical Textbook, each remedy consists of equal amounts (100 g) of their medicinal plant components.

Each powdered remedy (500 g) was individually macerated with 1000 mL of 95% ethanol at room temperature for 7 days. The extracts were then filtered through filter paper (Whatman No. 1) to remove solid plant material and the solvent was removed using a rotary evaporator and kept at 55 °C until the collected filtrates were completely dried. Yields (%; w/w) of each extracts calculated as the ratio of the weight of the extract to the weight of the recipe powder are reported in Table 2. The dried ethanol extracts were re-dissolved in dimethylsulfoxide (DMSO; Merck, Germany) to a stock concentration of 40 mg/ml and kept at −20 °C until use.

Phytochemical screening tests

Qualitative phytochemical screening analysis of the Thai ancient remedy extracts was done to identify the presence of antibacterial related-secondary metabolites, including alkaloids, terpenoids, and phenolics using Dragendorff's reagent, Liebermann-Burchard reagent, and ferric chloride reagent, respectively [12].

Tested pathogens

S. aureus ATCC25923, clinically isolated methicillin resistant *S. aureus* (MRSA NPRC R003-R005), *Staphylococcus epidermidis* ATCC 35984, coagulase negative staphylococci (CNS; NPRC 301 and 308) and coagulase positive staphylococci (CPS; NPRC 506-507) isolated from acne lesions, *P. aeruginosa* ATCC 10145, clinically isolated multidrug resistant (MDR) *P. aeruginosa* (2097 and 5351), MDR *A. baumannii* (NPRCAB002, 004, 005, and 034), *E. coli* ATCC 25922, MDR *E. coli* (2746-08 and 2809-08), and *E. coli* O15:H7 RIMD 05091078 were used in this study. The tested microbial strains were provided by Natural Products Research Center and Department of Microbiology, Faculty of Science, Prince of Songkla University. Each isolate was maintained on a Trypticase soy agar (TSA Difco, France) slant at 4 °C and activated at 37 °C for 24 h with a TSA plate prior to any antimicrobial tests.

Assessment of minimum inhibitory concentration (MIC)

The extracts were tested against the selected bacteria strains for their inhibitory activity using a modified broth microdilution method according to Clinical and Laboratory Standard Institute [13]. An aliquot of 100 μL of two-fold serial dilutions of the extract (16–1000 μg/mL) prepared using Mueller-Hinton broth (MHB; Difco, France) as the solvent was distributed into a 96-well sterile microtitre plate (Nunc, Denmark). Then, 100 μL of an inoculated broth obtained from an overnight growth at 37 °C and subsequently diluted with fresh MHB to achieve a bacterial culture concentration corresponding to 10^6 CFU/ml was added to each well. The plate was incubated for 24 h at 37 °C and the bacterial growth was measured by recording the absorbance at 620 nm, using a microplate reader (Sunrise, Tecan, Switzerland).

A growth control and a blank control were taken using the inoculated broth added into 2.5% of DMSO and fresh MHB added into each concentration of the extract, respectively. The determination of the MICs of antibiotics including vancomycin and rifampicin for all the reference strains was simultaneously carried out.