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Evaluation of extracts prepared from 16 plants used in Yao ethnomedicine as potential anticancer agents

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ABSTRACT

Ethnopharmacological relevance: Medicines of the Yao ethnic group in China are a special branch of traditional Chinese medicine (TCM) and are well documented for use in disease prevention. According to an ethnopharmacological survey, there are 1392 species of medicinal plants that have been documented as Yao ethnomedicines and 104 of these species are used routinely. This study evaluated a partial collection of these 104 core plant species for their potential as anticancer agents.

Material and methods: A literature study of scientific journals and books in the local language was conducted. Based on an ethnopharmacological survey, 16 plant species widely used in Yao ethnomedicine were collected and 64 plant extracts were prepared from these plants. *in vitro* cytotoxicity screening was conducted with a panel of four human cancer cell lines, lung cancer A549, breast cancer BT20 and MCF-7, bone cancer U2OS. The potential toxicity of the extracts was evaluated using two normal human cell lines, human peripheral lung epithelial cells (HPL1A) and human umbilical vein endothelial cells (HUVEC). Additionally, the 10 extracts that demonstrated cytotoxicity in cancer cells with an IC_{50} of less than 25.0 μg/mL were examined for the ability to induce apoptosis in U2OS cells.

Results: The up-to-date information regarding the traditional uses, pharmacological and biological activities, as well as the chemical constituents of the 16 plants are presented. Extracts from all 16 plants showed cytotoxicity against one to four of the human cancer cell lines and the cytotoxic effects of extracts from *Melaleuca leucadendra*, *Stephania longa*, *Microsorium fortune* and *Bidens biternata* were demonstrated for the first time. The highest anticancer potential was observed for extracts prepared from *Melaleuca leucadendra Linn* against all tested cancer cells (BT20, A549, U2OS, and MCF7) with an IC_{50} range of 3.1–32.7 μg/mL. The selectivity index of the active samples varied from 0.1 to 25, and five extracts from *Bidens biternata*, *Wedelia calendulacea*, *Stephania longa* and *Achras zapota* showed significant selectivity against cancer cell lines versus normal cell lines. All tested extracts induced apoptosis in U2OS cells, and for the first time extracts from *Melaleuca leucadendra* and *Microsorium fortune* were shown to induce apoptosis.

Conclusion: We demonstrated the *in vitro* anticancer efficacy and safety of 16 medicinal plants that have been historically used in Yao ethnomedicine. This study provides evidence to assist the clinical practice of Yao ethnomedicine and the development of chemotherapeutic agents from extracts prepared from these plants.

1. Introduction

Cancer is a life threating disease that causes 63% of deaths world wide and is known to be the second leading cause of death in western countries according to the World Health Organization (WHO) (Alwan et al., 2010; Kashfi, 2013; Monteiro et al., 2014). Chemotherapy has been used for decades to cure cancer. However, chemotherapeutic drugs are known to exhibit severe toxicity against normal tissues and are affected by the development of drug resistance. Due to these limitations of cancer therapy, there is an urgent need to develop new chemotherapeutic drugs (Efferth and Konkimalla, 2008; Tan et al., 2011). Medicinal plants have been used for many generations and play

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Table 1
Summary of the collection date, venue and plant part(s) of 16 medicinal plants used in Yao ethnomedicine.

	Name of the plant	Genus	Family	Plant part(s)	Collection date	Collection venue	Vochure #
1	*Garcinia paucinervis* Chun et How	*Garcinia*	*Clusiaceae*	Stem, branch, leaf	Jun 2009	GBGMP	200906001
2	*Elephantopus scaber* Linn.	*Elephantopus*	*Asteraceae*	Branch, leaf	Jun 2010	Jinxiu County, Guangxi	201006002
3	*Evodia lepta* (Spreng.) Merr.	*Evodia*	*Rutaceae*	Branch, leaf	Jun 2010	Jinxiu County, Guangxi	201006004
4	*Achras zapota* Linn.	*Achras*	*Sapotaceae*	Branch, leaf	May 2010	Lipu County, Guangxi	201005007
5	*Stephania longa* Lour.	*Stephania*	*Menispermaceae*	Whole plant	Jul 2012	Nanning city, Guangxi	201207009
6	*Crocosmia crocosmiflora* (Nichols.) N. E. Br.	*Crocosmia*	*Iridaceae*	Whole plant	Jul 2012	Nanning city, Guangxi	201207011
7	*Pandanus tectorius* Sol.	*Pandanus*	*Pandanaceae*	Aerial parts	Oct 2010	Wuzhou city, Guangxi	201010012
8	*Euphorbia hirta* Linn.	*Euphorbia*	*Euphorbiaceae*	Whole plant	Jun 2011	Yulin city, Guangxi	201106014
9	*Bidens biternata* (Lour.) Merr.	*Bidens*	*Asteraceae*	Whole plant	Jun 2010	Jinxiu County, Guangxi	201006016
10	*Cyperus rotundus* L.	*Cyperus*	*Cyperaceae*	Whole plant	Aug 2009	Wuzhou city, Guangxi	200908017
11	*Mosla caualeriei* Levl.	*Mosla*	*Labiatae*	Aerial parts	Aug 2011	Jinxiu County, Guangxi	201108021
12	*Bidens pilosa* L.	*Bidens*	*Asteraceae*	Whole plant	Jul 2011	Nanning city, Guangxi	201107022
13	*Wedelia calendulacea* Less.	*Wedelia*	*Asteraceae*	Whole plant	Jul 2011	Nanning city, Guangxi	201107027
14	*Uncaria macrophylla* Wall.	*Uncaria*	*Rubiaceae*	Branchs, leaves	Jun 2012	Guilin city, Guangxi	201206028
15	*Sarcandra glabra* (Thunb.) Nakai	*Sarcandra*	*Chloranthaceae*	Whole plant	Sep 2011	Lipu County, Guangxi	201109030
16	*Melaleuca leucadendra* Linn.	*Melaleuca*	*Myrtaceae*	Stem	May 2012	Nanning city, Guangxi	201205034

an integral role in primary health care in East Asia (Efferth et al., 2007). Attention to medicinal plants had begun to decrease due to the progress of chemical synthetic drugs, however, in the past two decades, interests in the plants used in traditional Chinese medicine (TCM) has risen in the western scientific community as new medicines have been derived from medicinal plants for the treatment of several major diseases (Chen and Liang, 2010; Efferth et al., 2007).

Numerous studies on plant extracts or individual bioactive compounds derived from medicinal plants have demonstrated promise for treating cancer. These extracts or compounds can kill cancer cells through several mechanisms, such as enhancing the immune system, inducing cell apoptosis, reversing multidrug resistance (MDR) and inhibiting angiogenesis (Balunas and Kinghorn, 2005; Ruan and Zhou, 2006). However, the anticancer mechanisms of TCM preparations are complicated, and specific mechanisms have not yet been fully determined (Ruan and Zhou, 2006). Although many studies have demonstrated that compounds derived from TCMs have great promise in cancer treatment, there is an urgent need to investigate their safety. Despite the fact that they are derived from natural sources, TCMs can have toxic effects since it is difficult to achieve biological activities without side effects (Chiu et al., 2009; Ruan and Zhou, 2006). For instance, studies on anticancer agents extracted from *Selaginaella doederleinii* reported that the agents inhibited the DNA polymerase in the human body and caused severe bone marrow suppression (Katsuhiko et al., 1989; Pan et al., 2001). Extracts from *Trypterigium wilfordii* Hook.f. (Thunder God Vine) demonstrated a promising anticancer activity, however, the extracts caused serious side effects when used at high concentration, including headaches, infertility, diarrhea and nausea (Shamon et al., 1997). Therefore, it is essential to evaluate the selectivity of extracts or the major constituents of herbal medicines in cancer cells versus normal cells to better understand their safety when investigating potential anticancer agents.

The Yao ethnic minority is an ancient ethnic group that has a total population of 2.6 million and is mainly distributed in the mountains of the Guangxi, Guangdong and Guizhou provinces in China and Yao ethnomedicine is a special branch of TCM. According to an ethnopharmacological survey, there are 1392 species of medicinal plants that have been used as Yao ethnomedicine and 104 of these species are used routinely. These 104 species, which belong to 84 genera of 50 families, constitute the core of the Yao minority healthcare system (Dai, 2009; Dai and Cui-chang, 1998; Li et al., 2006; Long and Li, 2004). Long and Li (2004) and Li et al. (2006) reported the ethnobotanical studies on the medicinal plants of Yao medicine, including the herbs that were used for medicinal baths, and recorded 66 species that belong to 61 genera of 43 families.

In this study, we collected a total of 16 plant species, *Garcinia paucinervis, Elephantopus scaber, Evodia lepta, Achras zapota, Stephania longa, Crocosmia crocosmiflora, Pandanus tectorius, Euphorbia hirta, Bidens biternata, Cyperus rotundus, Microsorium fortuni, Bidens pilosa, Wedelia calendulacea, Uncaria macrophylla, Sarcandra glabra* and *Melaleuca leucadendra* from the 104 core species routinely used in Yao medicine from the mountains of Guangxi, China. This effort took two years as some of the plants are very rare and often grow on the mountain cliffs, which makes them extremely difficult to collect. These plants have been used mostly for detoxification, expelling wind and dampness, or promoting and regulating blood circulation (Jia and Li, 2005b). The traditional uses of these plants have been passed down orally from generation to generation by the Yao ethnic minority people or have been recorded in local herbal books. We conducted a literature study of scientific journals as well as books written in the local language and present here the up-to-date information regarding the traditional uses, pharmacological and biological activities as well as the chemical constituents that have been isolated from these 16 plants. For each plant, four fractions (petroleum ether, ethyl acetate, ethanol and water) were prepared and tested against several cancer and normal cells lines to characterize their in vitro cytotoxicity and selectivity. This study enhances our understanding of Yao ethnomedicine and provides evidence to ensure its safety and efficacy in clinical practice as well as identifying potential new sources for the development of anticancer agents.

2. Materials and methods

2.1. Plant materials

The plant species were collected throughout the Guangxi Autonomous Region, China. Plants were characterized by Dr. Yimin Zhao and Dr. Xueyan Huang at the Guangxi Botanical Garden of Medicinal Plants (GBGMP). Which part(s) of the plant to collect (e.g. leaves, barks, stems, roots or whole plant) was based on their use in TCM. A summary of the collection date, venue and plant part(s) is shown in Table 1. Voucher specimens were deposited and identified at the GBGMP.

2.2. Preparation of plant extracts

Plant samples were dried and ground into powder using an electric blender. Each dried plant sample was extracted with petroleum ether and reflux. The solubilized material was dried down to generate the petroleum ether fraction (A). The remaining undissolved material was then extracted with ethyl acetate and reflux. The solubilized material was dried down to generate the ethyl acetate fraction (B). The