

Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang

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Abstract

Condensed tannins (CTs) are one of the promising compounds due to their potentially health-promoting qualities. In this study, CTs were extracted from a *Leucaena leucocephala* hybrid-Rendang and subjected to various biological studies including antioxidant (using Ferric reducing antioxidant power (FRAP), DPPH and ABTS radical scavenging assay), anti-microbial (against different pathogens) and cytotoxic activities (toward human breast adenocarcinoma (MCF-7), human colon carcinoma (HT29), human cervical carcinoma (HeLa) and human liver carcinoma (HepG2) cell lines) in cancer cells through *in vitro* experiments. The structural characteristics and purity of CTs extract were determined using ¹³C NMR. The results showed that CTs exhibited higher *in vitro* antioxidant activities (2257.12 ± 80.55 mg TEAC/g extract, 605.3 ± 1.82 mg TEAC/g extract and 1014.03 ± 1.20 mg TEAC/g extract in FRAP, ABTS and DPPH assay, respectively) and demonstrated anti-microbial activities toward selected Gram's positive and Gram's negative bacteria tested with MIC and MBC value at 6.25–50 mg/mL. Furthermore, among other selected cancer cells, CTs also demonstrated cytotoxic activity toward human breast cancer cells (MCF-7) (IC₅₀ = 38.33 ± 2.08 μg/mL). Characteristic of apoptosis such as cell shrinkage, nuclear condensation and apoptotic bodies were shown in MCF-7. These preliminary investigations have provided scientific rationale to use CTs as an alternative therapy for various oxidative and inflammatory associated diseases.

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1. Introduction

Since natural product-derived drugs frequently seem to be less toxic and more effective, identification and investigation of antioxidant, antimicrobial and anti-cancer agents from natural substances have been one of the research interests in recent years. Antioxidants scavenge variety of free radicals and reactive oxygen species and it can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases [1].

Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, Alzheimer's disease, Parkinson's disease, etc. Although several modern drugs are used to treat this type of disorder, their prolonged use may cause severe adverse side effects on chronic administration [2]. Cancer is the largest single cause of death in both men and women. Cancer is a class of diseases characterized by out of control of cell growth. There are different types of cancer, and each is classified by the type of cell that is initially affected. Cancer harms the body when altered cells divide uncontrollably to form lumps or masses of tissue called tumors except for leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream.

Breast cancer is the most common cancer in women followed by cervical cancer; with about more than 55% of breast cancer related deaths occur in the developing world. The incidence of

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this disease is increasing in both industrialized and developing countries [3]. Considerable progress has been made in treating breast cancer through surgery, radiotherapy, chemotherapy, and hormone therapy [4]. However, those carcinomas that do not express the estrogen remain generally resistant to therapy. Almost all cases of cervical cancer were from persistent infection with one of about 15 genotypes of carcinogenic human papillomavirus (HPV) [5]. Cases are often detected at late stages due to non-existent or inadequate screening, and the standard treatment options are often absent or unaffordable. Colon cancer is cancer of the large intestine. Most cases of colon cancer begin as small, noncancerous (benign) clumps of cells called adenomatous polyps and over time, some of these polyps become colon cancers. Meanwhile liver cancer is one of the major causes of malignancy-related deaths worldwide, and its incidence is on the rise. Typical treatment approaches to liver and colon cancer include surgery, radiotherapy, chemotherapy and transplantation but cure rates are not satisfactory [6]. To date, many anticancer drugs have been developed and applied by clinical doctors but recently, resistance to anticancer drugs was discovered; therefore, there is a need to develop new anticancer agents with minimum side effects.

Leucaena leucocephala hybrid-Rendang, which is known as the ‘miracle tree’ available in large quantities and abundant resources as well underutilize in Malaysia. This tree is a thornless tree which may grow up to 18 m and has a wide variety of uses, especially as a protein supplement for animals [7]. In Malaysia, a *Leucaena* hybrid, *L. leucocephala* hybrid-Rendang, which was produced from crossing *L. leucocephala* and *Leucaena diversifolia* [8], was found to have high condensed tannin content and low digestibility [9]. Condensed tannins (CTs), also known as proanthocyanidins, are polymers of 2–50 (or more) flavonoid units that are joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis and most CTs are water soluble [10]. Condensed tannins health benefits extend far beyond their antioxidant properties and also anti-inflammatory [11], anti-asthmatic [12], anticancer [13], anti-viral, anti-carcinogenic, anti-allergy, antimicrobial, antihypertension and cardiovascular system-protective [14]. Condensed tannins help wounds heal, reduce the pain from pancreatitis, reduce insulin resistance in diabetics, help protect from drug toxicity and also can help lower the levels of low-density lipoproteins, or the “bad” cholesterol. Antioxidants also decrease the oxidation of low density lipoproteins (LDL), which may lead to the buildup of plaque on the walls of arteries. Most of the previous study was done on reduction of methane and volatile fatty acid production in rumen digestion system using condensed tannins extract from *L. leucocephala* [15,16]. Thus, this study was conducted due to unexplored of condensed tannins from *L. leucocephala* on antioxidant, antimicrobial and cytotoxic activities.

The principal objective of the current exposition was therefore, to utilize and investigate the biological activities of CTs extract from *L. leucocephala* hybrid-Rendang including antioxidant using ferric reducing antioxidant power (FRAP), DPPH radical scavenging assay and ABTS radical scavenging assay, antimicrobial against *Staphylococcus aureus* (MRSA),

Staphylococcus aureus, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Acinetobacter anitratus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Serratia marcescens*, *Enterococcus faecium*, *Streptococcus faecalis*, *Candida albicans*, *Candida tropicalis*, and *Aspergillus niger* and cytotoxic activities toward human breast adenocarcinoma (Mcf-7), human colon carcinoma (HT29), human cervical carcinoma (HeLa) and human liver carcinoma (HepG2) cell lines whereas purity of the compound were confirmed by ¹³C NMR spectroscopy. These preliminary investigations will provide scientific rationale to use CTs from *L. leucocephala* as an alternative therapy for various inflammatory associated diseases.

2. Materials and methods

2.1. Chemicals

Acetone and sodium carbonate (Na₂CO₃) were obtained from R&M chemicals, diethyl ether, gallic acid and ethanol were purchased from Merck KGaA (Darmstadt, Germany), potassium ferricyanide, iron(III) chloride (FeCl₃), potassium persulphate were purchased from Pronadisa Chemie Brunschwig AG (Switzerland) trichloroacetic acid (TCA), Trolox (2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picryl hydrazyl (DPPH), nystatin, ampicillin, streptomycin, penicillin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA), nutrient agar (NA), Potato Dextrose Agar (PDA), Sabouraud agar (SDA) were purchased from Difco (BD, USA), RPMI 1640 and fetal bovine serum were purchased from PAA Laboratories GmbH (Pasching, Austria). Methanol was purchased from Thermo Fisher Scientific Inc. (United State) and Folin–Ciocalteu reagent was purchased from Fluka Analytical, Sigma–Aldrich Co. (St. Louis, MO, USA). All other reagents were of analytical and HPLC grades.

2.2. Plant material

Young leaves (3–4 layers from shoot) of *L. leucocephala* hybrid-Rendang was collected from a farm at Universiti Putra Malaysia. These plant materials were identified by our botanist, Dr Shamsul Khamis, Institute of Bioscience, Universiti Putra Malaysia and same material was stored at Herbarium for further identification.

2.3. Extraction and purification of condensed tannin

The leaves were cut into small pieces; freeze dried and then ground using a 0.5 mm sieve. The condensed tannins from the ground leaves were extracted as previously described [17]. Briefly, 5 g of ground leaves was soaked in 200 mL aqueous acetone solution (70%, v/v) for 20 min. After centrifugation (3500 × g for 10 min), the supernatant was filtered under vacuum to remove any particulate plant residues and washed with diethyl

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