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Research paper

Enzyme inhibition, antioxidant and immunomodulatory activities, and brine shrimp toxicity of extracts from the root bark, stem bark and leaves of *Terminalia macroptera*

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ABSTRACT

Ethnopharmacological relevance: The root bark, stem bark and leaves of *Terminalia macroptera* have been traditionally used against a variety of ailments such as wounds, hepatitis, malaria, fever, cough, and diarrhea as well as tuberculosis and skin diseases in African folk medicine. Boiling water extracts of *Terminalia macroptera*, administered orally, are the most common preparations of this plant used by the traditional healers in Mali. This study aimed to investigate the inhibition of the activities of α-glucosidase, 15-lipoxygenase and xanthine oxidase, DPPH scavenging activity, complement fixation activity and brine shrimp toxicity of different extracts obtained by boiling water extraction (BWE) and by ASE (accelerated solvent extraction) with ethanol, ethanol–water and water as extractants from different plant parts of *Terminalia macroptera*.

Materials and methods: 27 different crude extracts were obtained by BWE and ASE from root bark, stem bark and leaves of *Terminalia macroptera*. The total phenolic and carbohydrate contents, enzyme inhibition activities (α-glucosidase, 15-lipoxygenase and xanthine oxidase), DPPH scavenging activity, complement fixation activity and brine shrimp toxicity of these extracts were evaluated. Principal component analysis (PCA) was applied for total biological activities evaluation.

Results: Several of the extracts from root bark, stem bark and leaves of *Terminalia macroptera* obtained by BWE and ASE showed potent enzyme inhibition activities, radical-scavenging properties and complement fixation activities. None of the extracts are toxic against brine shrimp larvae in the test concentration. Based on the results from PCA, the ASE ethanol extracts of root bark and stem bark and the low molecular weight fraction of the 50% ethanol–water extract of leaves showed the highest total biological activities. The boiling water extracts were less active, but the bark extracts showed activity as α-glucosidase inhibitors and radical scavengers, the leaf extract being less active.

Conclusion: The observed enzyme inhibition activities, radical scavenging properties and complement fixation activities may explain some of the traditional uses of this medicinal tree, such as in wound healing and against diabetes.

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1. Introduction

Terminalia macroptera Guill. & Perr. (Combretaceae) is a tree that mostly grows in West Africa, and occasionally as far as Sudan and Uganda. The root bark, stem bark and leaves of the tree are used frequently in traditional African folk medicine. *Terminalia macroptera* is used against a variety of ailments such as wounds, hepatitis, malaria, fever, cough, diarrhea as well as tuberculosis and skin diseases (Diallo et al., 2002; Sanon et al., 2003; Pham et al., 2011a). The stem bark and leaves are most commonly used against sores and wounds, pain, cough, tuberculosis and hepatitis (Pham et al., 2011a). The roots are used against hepatitis, gonorrhea and various infectious diseases, including *Helicobacter pylori*-associated diseases (Silva et al., 1996, 1997, 2000, 2012; Pham et al., 2011a). Flavonoids (Nongonierma et al., 1987, 1988, 1990), triterpenoids (Conrad et al., 1998, 2001a), ellagitannins (Silva et al., 2000; Conrad et al., 2001b;

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Pham et al., 2011b) and other phenolics (Conrad et al., 2001a) have been identified from different parts of *Terminalia macroptera*.

A survey of the use of *Terminalia macroptera* by traditional healers in Mali indicated that diabetes was one of the illnesses mentioned (Pham et al., 2011a). Diabetes is a common metabolic disease characterized by abnormally high plasma glucose levels. α-Glucosidase is a key enzyme which catalyzes the final step in the digestive process of carbohydrates in mammals (Anam et al., 2009). An α-glucosidase inhibitor can reduce postprandial plasma glucose levels and suppresses postprandial hyperglycemia (Gao et al., 2008; Adisakwattana et al., 2011).

15-Lipoxygenase (15-LO) is an enzyme present in multiple systems that reacts with polyunsaturated fatty acids, producing active lipid metabolites which are involved in many diseases such as cancer, atherosclerosis and diabetes (Schneider and Bucar, 2005; Dobrian et al., 2011). Xanthine oxidase (XO) is a prooxidative enzyme that generates reactive oxygen species (ROS) in vascular cells (Pacher et al., 2006). Inhibition of 15-LO and XO may reduce the production of ROS which cause oxidative stress. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a frequently used method to estimate antioxidant capacities in extracts and naturally occurring compounds.

The complement system is an important part of the human immune defense, acting as a primary defense against bacterial invasions and viral infections. Complement fixating activity of polysaccharides from plants has previously been shown to be indicative of effects on the immune system (Michaelsen et al., 2000; Inngjerdingen et al., 2013).

Brine shrimp (*Artemia salina*) larvae have been used for over 30 years in toxicological studies (Meyer et al., 1982). To our knowledge, only the toxicity of crude extracts (dichloromethane, methanol, ethanol and butanol) and some isolated compounds from leaves of *Terminalia macroptera* has been reported previously (Pham et al. 2014); it was thus of interest to investigate the toxicity of other extracts from this tree.

Water decoctions of *Terminalia macroptera*, administered orally, are the most common preparations used by the traditional healers in Mali (Pham et al., 2011a). Thus, the boiling water extracts (BWE) would be expected to contain bioactive compounds present in the plant material. Traditionally in laboratory studies, low molecular weight and lipophilic compounds are extracted from plant material by the Soxhlet extraction method. Accelerated solvent extraction (ASE) for these types of compounds was first described by Ezzell et al. (1995), and it has grown steadily in use since that time (Richter and Raynie, 2012). Under elevated temperature and pressure, an extraction solvent can be used above its boiling point but still remain in the liquid state, thus improving the kinetics of the extraction process and leading to a significant decrease in solvent consumption and extraction time (Wang et al., 2010). ASE has been applied for extracting components from environment samples, biological materials, plant materials, dietary compounds, feeds, and food. It was thus of interest to compare the activities of bioactive extracts from medicinal plant by ASE and BWE.

Using leaves as a source of traditional medicines will reduce serious damage to the tree compared to the use of root and stem bark. Therefore, in this study, ASE and BWE were employed to obtain different extracts from leaf, root bark and stem bark from *Terminalia macroptera*. The aims of this study are comparing the enzyme inhibition activities, antioxidant activities, immunomodulating activities and toxicities of extracts by ASE with the conventional method (BWE), as well as to elucidate whether the activities differ among different plant parts. While some extracts and compounds from the leaves of this plant have been investigated for some biological activities (Pham et al., 2014), the activities of bark extracts, the activities of different extracts, and statistical comparison between different extracts have to our knowledge not been reported previously.

2. Materials and methods

2.1. Plant material

Leaves, root bark and stem bark of *Terminalia macroptera* were collected in Mali and identified by the Department of Traditional Medicine (DMT), Bamako, Mali. A voucher specimen is deposited at the herbarium of DMT (Voucher no. 2468/DMT). The plant material was washed, cut into small pieces, dried and pulverized to a powder with a knife mill (Brabender, Duisburg, Germany).

2.2. Extraction

2.2.1. BWE, boiling water extraction

BWE was carried out in the way traditional healers in Mali make water decoctions. Powdered root bark, stem bark or leaves (200 g of each) were placed in a pot, and extracted twice with boiling distilled water (2 L followed by 1 L) for 30 min each time. The extracts were centrifuged and filtered through Whatman No. 1 filter paper. Some of the filtrates were lyophilized and kept for further studies. These lyophilized samples were named TRB, TSB and TL for root bark, stem bark and leaves, respectively. The rest of the filtrates were subjected to ultrafiltration (cut off 5000 Da), and the low molecular weight (LMW) fractions and high molecular weight (HMW) fractions were lyophilized. TRBL, TSBL and TLL were LMW fractions from water extracts of root bark, stem bark and leaves (Fig. 1). These fractions were assayed for total phenolic content (TPC), total carbohydrate content (TCC), inhibition of enzymes (α-glucosidase, 15-lipoxygenase, xanthine oxidase), DPPH scavenging, complement fixation assay and brine shrimp toxicity assay. For method descriptions, see below.

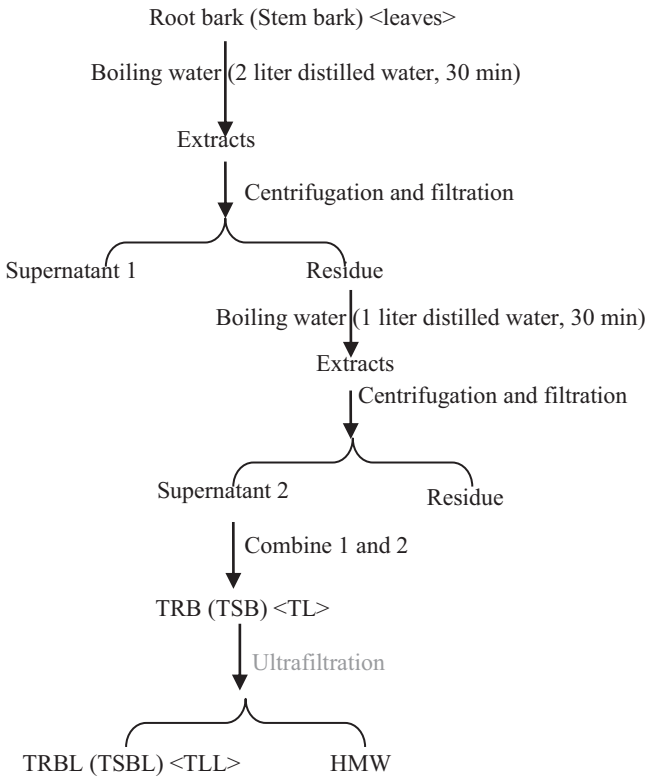

Fig. 1. Extraction scheme of crude extracts from root bark, stem bark and leaves of *Terminalia macroptera* by boiling water extraction (BWE).

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