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Complement activity of polysaccharides from three different plant parts of *Terminalia macroptera* extracted as healers do

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ABSTRACT

Ethnopharmacological relevance: Water decoctions of the root bark, stem bark and leaves of *Terminalia macroptera* are used by traditional healers in Mali to cure a wide range of illnesses, such as wounds, hepatitis, malaria, fever, cough and diarrhea as well as tuberculosis. Plant polysaccharides isolated from crude water extracts have previously shown effects related to the immune system. The aims of this study are comparing the properties of the polysaccharides among different plant parts, as well as relationship between chemical characteristics and complement fixation activities when the plant material has been extracted as the traditional healers do, with boiling water directly.

Materials and methods: Root bark, stem bark and leaves of *Terminalia macroptera* were extracted by boiling water, and five purified polysaccharide fractions were obtained by anion exchange chromatography and gel filtration. Chemical compositions were determined by GC of the TMS derivatives of the methyl-glycosides and the linkage determined after permethylation and GC–MS of the derived partly methylated alditol acetates. The bioactivity was determined by the complement fixation assay of the crude extracts and purified fractions.

Results: The acidic fraction TRBD-I-I isolated from the root bark was the most active of the fractions isolated. Structural studies showed that all purified fractions are of pectic nature, containing rhamnogalacturonan type I backbone. Arabinogalactan type II side chains were present in all fractions except TRBD-I-II. The observed differences in complement fixation activities among the five purified polysaccharide fractions are probably due to differences in monosaccharide compositions, linkage types and molecular sizes.

Conclusion: The crude extracts from root bark and stem bark have similar total activities, both higher than those from leaves. The root bark, leaves and stem bark are all good sources for fractions containing bioactive polysaccharides. But due to sustainability, it is prefer to use leaves rather than the other two plant parts, and then the dosage by weight must be higher when using leaves.

1. Introduction

Terminalia macroptera Guill. & Perr. (Combretaceae) is a tree which occurs widely in West Africa. In Mali *Terminalia macroptera* is used against a variety of ailments, about 31 different indications have been mentioned by the traditional healers in ethnopharmacological studies. 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 (Pham et al., 2011a; Silva et al., 1996, 1997, 2000, 2012). Flavonoids (Nongonierma et al., 1987, 1988, 1990), triterpenoids (Conrad et al., 1998, 2001a), ellagitannins (Pham et al., 2011b) and related phenolics (Conrad et al., 2001a, 2001b; Silva et al., 2000), have been identified from different parts of *T. macroptera*.

Water decoctions of *Terminalia macroptera*, administered orally, are the most common preparations used by the traditional healers

Abbreviations: AG-I, arabinogalactan type I; AG-II, arabinogalactan type II; Ara, arabinose; ASE, accelerated solvent extraction; BWE, boiling water extraction; Gal, galactose; GalA, galacturonic acid; Glc, glucose; GlcA, glucuronic acid; Man, mannose; RG-I, rhamnogalacturonan type I; Rha, rhamnose; Xyl, xylose

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in Mali (Pham et al., 2011a). Thus the boiling water extracts (BWE) should contain bioactive compounds present in the plant material. Plant polysaccharides isolated from crude water extracts have shown effects related to the immune system by different *in vitro* and *in vivo* test systems (Paulsen and Barsett, 2005). The chemical characteristics and biological activities of polysaccharides, especially those from plants used in the treatment of wounds, ulcer and cancer have been reported (Austarheim et al., 2012a; Lin et al., 2013; Samuelsen et al., 1996; Yamada and Kiyohara, 1999; Zong et al., 2012).

The root bark, stem bark and leaves of the tree are used frequently in traditional African folk medicine. If the root bark from a tree is collected this can lead to serious damages to the tree being greater than if the stem bark or leaves are collected. Therefore, in this study, BWE was employed to extract polysaccharides from root bark, stem bark and leaves from *Terminalia macroptera* for testing if all plant material contains bioactive polysaccharides of more or less equal bioactivity. Generally, studies of plant polysaccharides take place after the plant material has been extracted with organic solvent in order to remove lipids and low molecular weight compounds. The aims of this study are comparison of the polysaccharides properties among different plant parts, as well as relationship between chemical characteristics and complement fixation activities when the plant material has been extracted as the traditional healers do, with boiling water directly. Crude polysaccharide extracts were obtained and further purified, the chemical characteristics and complement fixation activities of polysaccharide fractions were evaluated, and the results from the three different plant parts were compared.

2. Materials and methods

2.1. Plant material

The root bark, stem bark and leaves of *Terminalia macroptera* were collected in Mali, and identified by the Department of Traditional Medicine (DMT), 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 fine powder by a mechanical grinder.

2.2. Extraction of polysaccharides

BWE was carried out in the way traditional healers in Mali make water decoctions. 200 g of powdered root bark, stem bark and leaves were weighed and 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. The filtrates were combined and subjected to ultrafiltration with cut off 5000 Da, and the high molecular weight (HMW) fraction was subjected to dialysis in a dialysis tube with cut off 3500 Da, lyophilized and kept for further studies. The crude, dialyzed, water extracts were denominated TRBD for root bark extracts, TSBD for stem bark extracts and TLD for leaves extracts (Fig. 1). These fractions were subjected to monosaccharide determination, evaluation of presence of starch and the complement fixation assay; methods see below.

2.3. Fractionation and characterization of polysaccharides

The crude extracts showing high activity in the complement assay were further fractionated by anion exchange and gel filtration. All purified fractions were subjected to determination of their chemical and biological characteristics.

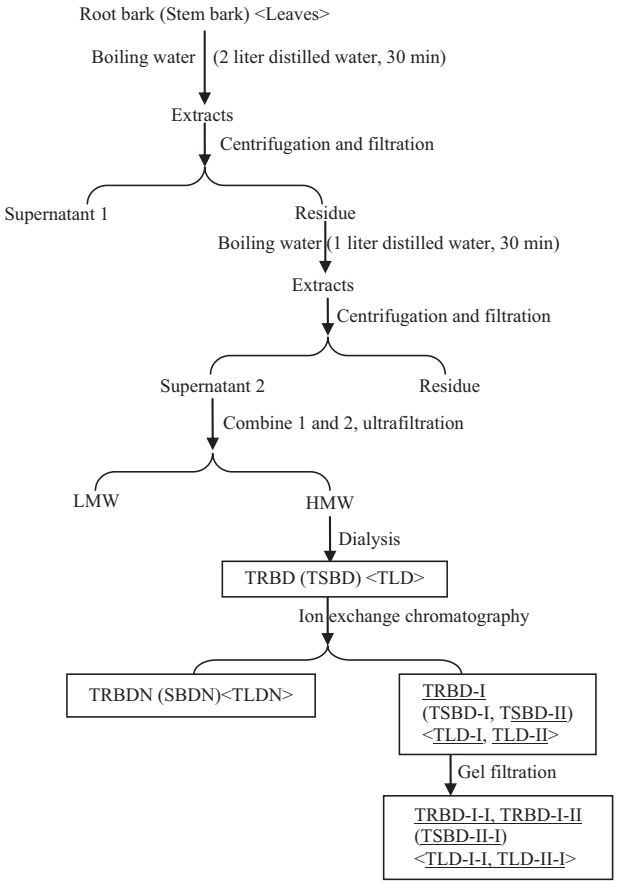

Fig. 1. Extraction and fractionation scheme of polysaccharides extracted with boiling water (BWE) from root bark, stem bark or leaves of *Terminalia macroptera* (underlined acidic fractions showed high complement fixation activity and were fractionated for further studies).

2.3.1. Ion exchange chromatography and gel filtration

The crude extracts from BWE were filtered through 0.45 μm filters and applied to an anion exchange column packed with ANX Sepharose™ 4 Fast Flow (high sub) (GE Healthcare). The neutral fractions were eluted with distilled water at (2 mL/min), while the acidic fractions were eluted with a linear NaCl gradient in water (0–1.5 M) at 2 mL/min. The carbohydrate elution profiles were monitored using the phenol-sulfuric acid method (Dubois et al., 1956). The related fractions were pooled, dialyzed at cut-off 3500 Da against distilled water for removal of NaCl, and lyophilized.

The acidic fractions marked in Fig. 1 were dissolved in elution buffer (10 mM NaCl), filtered through a Millipore filter (0.45 μm), and subjected to gel filtration after application on a Hiload™ 26/60 Superdex™ 200 prep grade column (GE Healthcare) combined with the Äkta system (FPLC, Pharmacia Äkta, Amersham Pharmacia Biotech). Fractions were pooled based on their elution profiles, as determined by the phenol-sulfuric acid method, dialyzed and lyophilized.

2.3.2. Determination of monosaccharide composition

The monosaccharide compositions of the crude extracts and purified fractions were determined by gas chromatography of the trimethylsilylated (TMS) derivatives of the methyl-glycosides obtained after methanolysis with 3 M hydrochloric acid in anhydrous methanol for 24 h at 80 °C (Austarheim et al., 2012b; Barsett et al., 1992; Chambers and Clamp, 1971). Mannitol was used as an internal standard. The TMS derivatives were analyzed by