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Screening of antioxidant activities of polysaccharides extracts from endemic plants in Gabon

Line Edwige Mengome[a,b], *Aline Voxeur*[b], *Jean Paul Akue*[c,*], *Patrice Lerouge*[b]

[a]Institut de Pharmacopée et de Médicine Traditionnelle, Libreville, Gabon
[b]Laboratoire Glyco-MEV EA 4358, IRIB, Université de Rouen, 76821 Mont-Saint-Aignan, France
[c]Centre International de Recherches Médicales de Franceville, BP 769, CIRMF, Franceville, Gabon

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ABSTRACT

The usage of plants as food, cosmetics or medicinal products is common throughout the world, particularly in the traditional medicines of Africa, Asia, and South America. Despite this long-standing use, many properties of plant constituents, including their capacity to inactivate reactive oxygen species, remain unknown today. In this context, polysaccharides were isolated from barks, leaves or stems of endemic plants of Gabon by sequential extractions of crude cell walls with oxalate and KOH. Analysis of fractions indicated that pectic extracts are mainly composed of HG and RG-I in various proportions whereas hemicellulose fractions are composed of both XXXG-type xyloglucans and (1,4)-xylans substituted by 4-O-Me GlcUA residues. Antioxidant activities of these plant extracts were evaluated by monitoring their free radical scavenging activity of 2-2-diphenyl, 1-picrylhydrazyl, iron reducing capacity and metal chelating activity. Pectin from four extracts presented antioxidant activity in the DPPH assay compared to ascorbic acid (AA). These extracts also exhibited high ferric iron reducing powers compared to AA but low ferrous ion-chelating properties compared to EDTA. These activities were concentration-dependent. In contrast, commercially available pectins, xyloglucan and arabinoxylan were found to be inactive in both assays. The free scavenging activity of these molecules disappeared after enzymatic digestion and saponification, whereas the iron reducing power partially remains after these two treatments. The fact that all pectins and hemicelluloses from the three plants reacted differently suggests that specific structural motifs are involved for generation of antioxidant activity

1. Introduction

Polysaccharides are the major component of the plant cells wall (90%) participating in the defense, regulation and rigidity of plant cells (Caffall & Mohnen, 2009; Liepman et al., 2010). They are classified into three main classes of polysaccharides: cellulose, hemicelluloses and pectins (Caffall & Mohnen, 2009; Liepman et al., 2010). Xylans and xyloglucans are the main hemicelluloses of plant cell walls. Commercially extracted pectins are complex acidic polysaccharides of the cell wall containing two main distinct domains: homogalacturonan (HG) and rhamnogalacturonan-I (RG-I). HG is a polymer of repeated units of α(1-4)-D-GalUA that can be methylesterified and acetylesterified. RG-I consists of the repeating disaccharide

*Corresponding author. Tel.: +241 677092.
E-mail address: jpakue@yahoo.fr (J.P. Akue).

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α(1-4)-D-GalUA-α(1-2)-L-Rha substituted with a wide variety of side chains attached to the rhamnosyl residues, ranging from monomers to large oligosaccharides such as β(1–4)-D-galactan and α(1–5)-L-arabinan (Caffall & Mohnen, 2009).

The usage of plants as food, cosmetics or medicinal products is common throughout the world (Yamada & Kiyohara, 1999; Shi, Sheng, Yang, & Hu, 2007), particularly in the traditional medicines of Africa, Asia, and South America where the two-third of the world's population has a low income per capita. Despite this long-standing use, many properties of plant constituents remain unknown today. Numerous studies have shown that extracts or purified polysaccharides are able to inactivate reactive oxygen species and significantly protect DNA chains or membranes from being damaged by hydroxyl radicals (Parij & Nève, 1996; Asker, Ahmed, & Ramadan, 2009; Gülçin, Mshvildadze, Gepdiremen, & Elias, 2006). Reactive oxygen species are unstable ($O_2^{\cdot}$, $HO^{\cdot}$, H_2O_2, NO^-, $ONOO^-$). They are found throughout the immune system and play important roles against pathogens (bacteria, viruse, *etc.*). In living organisms, free radical activities are regulated by enzymes (glutathione peroxidase, superoxide dismutase, *etc.*). They enable the inactivation of common mechanisms of lipid peroxidation (Gülçin, Huyut, Elmastas, & Aboul-Enein, 2010). Unfortunately, these enzymes have a short life span and their quantitative or qualitative deregulation induces oxidative stress, in turn inducing diseases such as cardiovascular diseases, diabetes, hypertension, respiratory disorders, cancers, neurological ageing, inflammation, skin irritations, Alzheimer and Parkinson diseases, epilepsy, fibroplasias, and atherosclerosis (Parij & Nève, 1996; Asker et al., 2009; Bursal & Gülçin, 2011; Gacche & Dhole, 2011). Antioxidants are able to inhibit the action of free radicals. However, these molecules are insufficient to entirely prevent radical-induced damages (Asker et al., 2009; Roy et al., 2011; Noipa, Srijaranai, Tuntulani, & Ngeontae, 2011). Intake of antioxidants as dietary supplements or in cosmetics has a great impact on health. Unfortunately, it has been shown that some synthetic antioxidants such as butylated hydroxyl toluene (BHT) or butylated hydroxyl anisole (BHA) have been suspected of being responsible for liver damage and carcinogenesis (Asker et al., 2009; Pan & Mei, 2011; Roy et al., 2011). *In vitro* studies have demonstrated that antioxidants from plants are sometimes more active than those obtained from chemical synthesis like BHT, BHA or vitamin E (Gordon & Weng, 1992; Gu & Weng, 2001; Valentao et al., 2002; Pyo, Lee, Logendrac, & Rosen, 2004; Gülçin, 2005; Asker et al., 2009; Roy et al., 2011). Recently, a vast body of literature has demonstrated that many non-toxic antioxidant compounds naturally occurring in plant sources are free radical or active oxygen scavengers (Gacche & Dhole, 2011). Although antioxidant activity is usually associated with low molecular weight compounds such as phenols, carotenoids and vitamins, an increasing amount of evidence highlights that some polysaccharides isolated from plants, herbs and fungi also exhibit antioxidant properties and low cytotoxicity (Liu, Ooi, & Chang, 1997; Gülçin et al., 2006; Asker et al., 2009).

The use of traditional medicine is wide-spread throughout the world and plants remain a large source of natural antioxidants that might serve as leads for the development of novel drugs (Liu et al., 2011). This paper therefore examines the potential antioxidant activity of pectin and hemicellulose extracts isolated from cell walls of endemic plants in Gabon. Here, different *in vitro* chemical assays were carried out to monitor the radical scavenging activity, ferric ion-reducing antioxidant power and ferrous ion-chelating activity of these plant extracts.

2. Material and methods

2.1. Chemicals

L-ascorbic acid, the stable free radical DPPH, 3-(2-pyridyl)-5,6-bis (4-phenylsulfonic acid)-1,2,4-triazine (ferrozine), and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck. EDTA, $FeCl_2$, $FeCl_3$, potassium ferricyanide and all other chemicals used were of analytical grade and obtained from either Sigma-Aldrich or Merck. Citrus pectin with DM85 and polygalacturonic acid from orange were from Sigma. Xyloglucan from tamarind seed and arabinoxylan from wheat were from Megazyme International Ireland.

2.2. Natural samples

Plants selected for this study (Table 1) were chosen initially as being endemic to Gabon according to Sosef et al. (2006). For their therapeutic virtues, we referred to Walker and Sillans (1961), Aubréville (1968), Aubreville and Leroy (1970), De Saint Aubin (1963) and Liben (1968). These plants were collected in the Estuaire and Ngounie regions of Gabon. The identification of the voucher specimens was confirmed by the National Herbarium of Gabon (HNG) and herbaria were made. Different parts were used for extraction: leaf; bark and stem. These plants parts were dried and crushed at the Institut de Pharmacopée et de Médecine Traditionnelle (IPHAMETRA) in Gabon. Then, the cell wall polysaccharides were extracted and characterized at the University of Rouen in France. The antioxidant activity of the samples was recorded at the Centre International de Recherches Médicales de Franceville (CIRMF) in Gabon.

Table 1 – List of plants endemic of Gabon investigated in this study.

Plants	Parts used	Family	Names used
Aphanocalyx microphyllus (Harms) Weiring	Barks	Fabaceae – Caesalpinioideae	AMB
Petersianthus macrocarpus (P. Beauv.) Liben	Barks	Lecythidaceae	PMB
Uvaria klainei Pierre ex Engl. & Diels	Leaves, stems	Annonaceae	UKL/UKS