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Characterisation of cell wall polysaccharides, arabinogalactans-proteins (AGPs) and phenolics of *Cola nitida*, *Cola acuminata* and *Garcinia kola* seeds

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ABSTRACT

Many Cola plant species are endemic to West and Central Africa. *Cola acuminata* and *Cola nitida* are used as masticatory when fresh, while the dried nuts are used for beverages and pharmaceutical purposes in Europe and North America. *Garcinia kola* seeds, that serve as a substitute for the true kola nuts, are used in African traditional medicine for the treatment of various diseases, including colic, headache and liver cirrhosis. Seeds extracts of *G. kola* are also known for their anti-inflammatory, antimicrobial and antiviral properties. To gain information on the chemical properties of the kolas, we have isolated and analyzed cell wall polysaccharides, arabinogalactan-proteins and phenolic substances from the seeds of the three kola species. The sugar composition of cell wall material of *C. acuminata*, *C. nitida* and *G. kola* revealed that Gal (up to 30%), Ara, GalA and Glc as the predominant monosaccharides, representing approximately 90% by mol of the total hydrolysable sugar present in this material. In Ammonium oxalate cell wall fraction, GalA was found to be the major sugar present in all kola species. In the alkali-soluble fraction, there were significant differences in the level of Glc and Gal. The level of Glc was high in *C. acuminata* and *C. nitida* while the level of Gal and Xyl were high in *C. nitida* and *G. cola*. Isolation and quantification of arabinogalactan-proteins demonstrate that *G. kola* seeds contained four to eight times more of these proteoglycans than the seeds of the other two species. Finally, analysis of soluble phenolic substances shows that caffeine and catechin were largely represented in *C. acumina* and *C. nitida* seeds, with caffeine accounting for ~50% of all soluble phenolics. These findings indicate that the three Kola seeds are highly enriched in pectins and proteoglycans and that *C. acuminata* and *C. nitida* can be used as a possible source of caffeine and catechin.

1. Introduction

Cola *sp* is a genus belonging to the sterculiaceae family and is endemic in Central and West Africa. The agronomic value of the plant lies in the nuts. Both *Cola acuminata* and *Cola nitida* are used as masticatory when fresh, while the dried nuts are used for beverages and pharmaceutical purposes in Europe and North America (Adeyeye, Asaolu, & Aluko, 2007; Atawodi et al., 2007). The nuts are eaten raw as a stimulant to resist hunger and fatigue (Atawodi, Mende, Pfundstein, Preussmann, & Spiegelhalder, 1995). *C. nitida* is easily distinguished by its nuts presenting two cotyledons whereas *C. acuminata* has three to six cotyledons. Unlike *C. acuminata*, *C. nitida* has three varieties based on colour; white, red and pink. The main area of cola growth and production in Cameroon is the rain forest zone.

Garcinia kola (Guttiferae) is a tree found in southern part of Cameroon and other West and Central African nations. It can serve as a substitute for the true cola nuts (*Cola acuminata*, *C. anomala* and *Cola nitida*). It has a bitter astringent taste when chewed, resembling that of raw coffee bean, followed by light sweetness. It is an economic and highly valued tree used extensively in African traditional medicine for the treatment of various diseases (Farombi, Tahnteng, Agbooola, Nwankwo, & Emerole, 2000). In the past decades, many research groups had investigated its chemical constituents and biological activities (Iwu, Igboko, Onwuchekwa, & Okunji, 1987; Okunji et al., 2007). The seed has been used in local medicine to relieve cough, colics, headaches, chest cold and hoarseness hence improving singing voice. It is used for the treatment of liver cirrhosis (Iwu, Igboko, Elekwa, & Tempesta,

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1990). *G. kola* seeds administered at 200 mg/kg body weight alter the oestrous cycle in rats, partly inhibiting ovulation and may produce duration dependent teratogenicity in foetal rats (Akpantah, Oremosu, Noronha, Ekanem, & Okanlawon, 2005). In addition, the seeds possess antidiabetic, and antihepatotoxic activities (Iwu, Igboko, Okunji, & Tempesta, 1993). They also enhance the flavour of some local beverages.

Plant cell walls are highly complex structures whose composition and physical properties vary depending on the cell type, plant species and stage of development (Harris, 2005; Harris & Smith, 2006). Nevertheless, all plant cell walls have a similar basic structure: they consist of a fibrillar phase of cellulose microfibrils set in a matrix. This matrix is composed mostly of polysaccharides (non-cellulosic polysaccharides) with a variety of different structures, structural proteins, glycoproteins, and phenolic components, such as lignin.

Non-cellulosic cell wall polysaccharides of higher plants represent a potential source of pharmacologically active polysaccharides. In traditional medicine, extracts of polysaccharides-containing plants are widely used for the treatment of skin problem, epithelium wounds and for mucous membrane irritation (Cho et al., 2000; Ye, So, Liu, Shin, & Cho, 2003). Moreover, several reports show that polysaccharides from different plants could be responsible for the effects associated with the healing of wounds. Some of these have an influence on the immune system and are often called immunomodulators when the complement system is involved (Diallo, Berit, Torun, & Terje, 2001; Ebringerová, Kardošová, Hromádková, Malovíková, & Hříbalová, 2002; Yang, Zhao, & Lv, 2008). Acidic pectin fractions extracted from Chinese herbs *Bupleurum falcatum* and from *Atractylodes lancea* have been shown to exhibit anti-ulcer and immune system modulatory activities (Yamada, Masumi, & Kiyohara, 1991; Yu, Kiyohara, Matsumoto, Yang, & Yamada, 2001).

Arabinogalactans-proteins (AGPs) are highly glycosylated proteins of the hydroxyl rich glycoprotein family. The carbohydrate moiety of these proteins represents 90–99% of molecular weight of the molecule, while the amino-acid backbone reaches around 1–10% (w/w) (Gaspar, Johnson, Mckenna, Bacic, & Shultz, 2001). The Carbohydrate moiety of AGPs is particularly rich in galactose and arabinose and is organized as arabinan and arabinogalactan chains linked to hydroxyprolin residues (Showalter, 2001). AGPs are widespread through the plant kingdom and seem to be implicated in diverse developmental processes such as differentiation, cell–cell recognition, cell adhesion, embryogenesis and programmed cell death (Lee et al., 2005; Mollet, Kim, Jauh, & Lord, 2002; Nguema-Ona et al., 2006; Vicré, Lerouxel, Farrant, Lerouge, & Driouich, 2004). AGPs from a number of crops and medicinal plants have been purified and their structure determined (Showalter, 2001; Yu et al., 2001). Both the intact AGP and its purified polysaccharide fraction arabinogalactan, are found to be highly water-soluble and possess a high degree of biocompatibility and used in a number of pharmaceutical and neutraceutical preparations (Alban, Classen, Brunner, & Blaschek, 2002). For instance, AGPs extracted from *Baptisia tinctoria* and *Echinacea pallida* roots have been shown to stimulate the proliferation and IgM-production of mouse lymphocytes (Classen, Thude, Blascheka, Wacka, & Bodinet, 2006; Thude, Classen, Blaschek, Barz, & Thude, 2006).

In this study, we report on the chemical composition of the cell wall polysaccharides, AGPs and phenolic substances of *C. acuminata, C. nitida* and *G. kola* seeds. This is to gain baseline information that could be related to the biological activities and food composition of the "kola" species.

2. Materials and methods

C. acuminata, C. nitida and *G. kola* seeds were purchased at the Mokolo market, Yaoundé, Cameroon. The identification of the voucher specimens of *C. acuminata, C. nitida* and *G. kola* was confirmed by the National Herbarium of Cameroon. The fresh mature seeds were ground in the laboratory before use.

2.1. Water-soluble polysaccharides and arabinogalactan-proteins: extraction and analysis

Crude water-soluble polysaccharides were extracted according to Schultz, Johnson, Currie, and Bacic (2000). Briefly, 5 g of fresh nuts were ground under liquid nitrogen and suspended in the extraction buffer (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 0.1% β-mercaptoethanol and 1% (w/v) and incubated at 4 °C for 4 h. The pellet was resuspended in a 5 mL of 50 mM Tris–HCl pH 8.0. The insoluble material was removed by centrifugation and supernatant was collected. The pellet was resuspended in additional 5 mL of 50 mM Tris–HCl pH 8.0. The supernatants were pooled and precipitated with ethanol 95° (4 vols) at 4 °C. The precipitate was freeze-dried, dissolved in 1 mL of 1% (w/v) NaCl and transferred to a 1.5 mL microcentrifuge tube and constituted total water-soluble polysaccharides.

AGPs were precipitated with the β-glucosyl Yariv reagent (β-GlcY) by mixing 1 mL of soluble polysaccharides with 1 mL of β-GlcY (2 mg/mL) in 1% NaCl and incubated at 4 °C, overnight. The insoluble AGP–Yariv complex was collected by centrifugation at 14,000*g* in a microcentrifuge for 1 h. The pellet was washed in 0.1 M NaCl and deionized water. Sodium hydrosulphide ($Na_2S_2O_4$) was added to a final concentration of 10% (w/v) to decompose β-GLcY. The samples were dialyzed extensively against water at 4 °C and freeze-dried. The dried AGPs were dissolved in 1 mL of 1% (w/v) NaCl.

AGPs quantification was done by glucosyl Yariv reagent binding in rocket gel electrophoresis. Briefly, electrophoresis was performed in 1% agarose gel containing 15 μM Yariv reagent for AGP precipitation as described by Ding and Zhu (1997) in Tris–glycine buffer (25 mM Tris, 200 mM glycine, pH 8.4) for several hours, until the rockets were well developed. Seven microliters of AGPs solutions of *C. acuminata*, *C. nitida* and *G. kola* and standard solution (AGP of gum arabic) (1 mg/mL) were deposited in each well. The concentration of AGP in the samples was estimated in relation to the peak area of gum arabic.

The AGP molecules were further characterized by mono-dimensional and by two-dimensional crossed electrophoresis (Girault, His, Andème-Onzighi, Driouich, & Morvan, 2000; Ray, Loutier-Bourhis, Condamine, Driouich, & Lerouge, 2004; Van Holst & Clarke, 1986). For mono-dimensional analysis, AGPs (~20 μg) were separated in 1% agarose gels with Tris–boric acid–EDTA buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.3). After electrophoresis, staining of AGPs was assayed by placing the gel in the Yariv (20 μM) solution of water/DMSO (19/1, v/v). For two-dimensional crossed electrophoresis, after mono-dimensional electrophoresis, the gels were run in the second dimension in a 1% agarose gel in Tris–glycine buffer, pH 8.4, supplemented with 15 μM Yariv reagent, which precipitated the AGPs as they moved towards the anode. For mono-dimensional and two-dimensional electrophoresis, de-staining of gels to remove unbound Yariv reagent was performed by overnight washing in 1% NaCl. As a result, characteristic precipitation profiles were obtained for each sample.

2.2. Cell wall extraction, isolation of pectin, hemicellulosic and cellulosic fractions

Fresh *C. acuminata, C. nitida and G. kola* seeds (8 g) were ground to a fine powder (under liquid N_2) and then suspended in 100 mL of boiling ethanol (80%, v/v) at 100 °C for 30 min to deactivate any enzymes present. The pellet was subjected to a series of extractions to remove lipids, polyphenols, and other

low-MW metabolites as previously described by Moore et al. (2006). Briefly, the residues were extracted overnight at room temperature with 100 mL of 90% DMSO, 24 h with methanol–chloroform (1:1; v/v), 24 h with methanol–acetone (1:1; v/v), and finally with acetone–water (4:1; v/v). The residue was air dried at 80 °C and the cell wall material (CWM) obtained was suspended in 50 mM acetate, pH 5.4. It was then destarched at 80 °C using a thermostable α-amylase and amyloglucosidase (EC 3.2.1.1; Megazyme International). CWM was used for the determination of monosaccharide composition by using gas chromatography (GC) and for the extraction of pectic and hemicellulosic wall polymers.

To extract the pectic and hemicellulosic fractions, CWM (1 g) was extracted twice with boiled ammonium oxalate at 0.5% for 1 h followed by incubation of the residue in 1 M KOH overnight at room temperature as described by Ray et al. (2004). The 1 M KOH hemicellulosic fraction was separated into a soluble (SF) and an insoluble fraction (InF). All extracts were centrifuged and dialyzed against water; alkaline extracts were acidified to pH 5 with acetic acid prior to dialysis. Each fraction extracted (Table 1) was gravimetrically analyzed before being hydrolyzed with TFA; insoluble material remaining after TFA hydrolysis of CWM was hydrolyzed with H_2SO_4 (cellulosic fraction). These hydrolysates were then analyzed for the presence of individual monosaccharides.

2.3. Monosaccharide composition analysis

The total carbohydrate content was determined by the phenol–sulfuric acid method using D-glucoses standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The carbohydrate content was expressed in mg (equivalent of glucose) per mg of material. Total UA content of CWM and water-soluble polysaccharide fractions were estimated according to the method of Blumenkrantz and Asboe-Hansen (1973). UA content was expressed in mg (equivalent of galacturonic acid) per mg of material.

Monosaccharide composition was performed according to Ray et al. (2004) and analyzed after trifluoroacetic acid hydrolysis. The generated methyl glycosides were converted into their TMS derivatives and separated by gas chromatography (GC) using inositol as the internal standard. Briefly, each fraction (3–10 mg) was hydrolyzed (2 M TFA, 110 °C, 2 h) and the liberated monosaccharides converted into methoxy sugars by incubation at 80 °C for 24 h in 1 M methanolic HCl. After sialylation at 80 °C for 30 min, samples were dried, dissolved in cyclohexane, and analyzed using a GC 3800 Varian gas chromatography system equipped with a DB1 capillary column and a flame ionization detector. A temperature program optimized for separation of the most common cell wall monosaccharides, specifically, Ara, Fuc, Gal, GalA, Glc, GlcA, Man, Rha, Xyl, as well as the internal standards inositol, was used. Chromatographic data were analyzed and integrated using Varian GC Star Workstation software with the quantity of each monosaccharide corrected according to its response factor.

Table 1
Yield of polysaccharide fractions isolated from *C. acuminata*, *C. nitida* and *G. kola* seeds. KOHA and KOHB represent soluble and insoluble components respectively, when extracted with KOH 1 M.

Fractions	*Cola acuminata*	*Cola nitida*	*Garcinia kola*
Water-soluble fractions	0.24 ± 0.04	0.16 ± 0.03	1.09 ± 0.12
CWM	49 ± 1.1	25 ± 0.7	42 ± 0.8
Ammonium oxalate fraction	15.51 ± 0.5	32 ± 1.5	39.52 ± 2.5
KOHA	14.93 ± 1.4	14 ± 1.2	4.76 ± 0.5
KOHB	15.30 ± 1.3	16 ± 1.5	2.85 ± 0.3
Insoluble residue	38.77 ± 2.5	28 ± 2.8	46.42 ± 3.1

2.4. Phenolic analysis

Extraction and quantitative measurement of phenolics were performed as described by Boudjeko et al. (2007). Total phenolic compounds were extracted twice using 0.1 M hydrochloric acid (HCl). One gram of fresh tissue was ground in 3 mL 0.1 M HCl. After 30 min incubation at room temperature, the ground material was centrifuged at 6000*g* for 30 min. The supernatant was removed and the precipitate resuspended in 3 mL 0.1 M HCl and incubated at room temperature for 15 min. After the second centrifugation, the supernatants were pooled to constitute the phenolic extract. The concentration of phenolic compounds was determined spectrophotometrically at 725 nm, according to the method of Marigo (1973), using the Folin–Ciocalteu reagent. Phenolic contents were expressed in mg equivalent of chlorogenic acid per g of fresh weight (Fw).

For qualitative analysis of phenolics, frozen tissues from *C. acuminata*, *C. nitida* and *G. kola* seeds were extracted with 80% aq. Methanol (MeOH) at 4 °C with continuous stirring and with acetone. The homogenate was centrifuged at 7000*g* for 3 min and the supernatants were stored at −20 °C until they were analyzed by high performance liquid chromatography (HPLC) using a Waters 600E HPLC (Waters, Milford, MA) equipped with a Waters 990 photodiode Array Detector and Millipore Software for data analysis. An efficient gradient of acetonitrile-*o*-phosphoric acidified bidistilled water (pH 2.6) was used with an Interchrom C18, 5-μm reversed phase column. Phenolics were identified on the basis of their retention time and their spectra in comparison with standards (*p*-coumaric acid, caffeic acid, synapic acid, catechin, eriodyctiol and epicatechin, from Sigma–Aldrich, France). When necessary, standards were co-injected to confirm the identity of certain compounds.

3. Results and discussion

3.1. Yield of polysaccharide fractions

Gravimetric analysis of lyophilized fractions recovered from a sequential fractionation (Table 1) yielded significant differences between these species in water-soluble fractions, ammonium oxalate fractions and 1 M KOH fractions. Water extracted more material (water-soluble-polysaccharides fractions) from *G. kola* (1.09 ± 0.12% of Fw) than from *C. acuminata* (0.24 ± 0.04% of Fw) and from *C. nitida* (0.16 ± 0.03% of Fw) samples. Furthermore, ammonium oxalate extracted more material in *G. kola* (39.52 ± 2.5% of CWM) than in *C. acuminata* (15.51 ± 0.5% of CWM) and *C. nitida* (32 ± 1.5% of CWM) (Table 1).

In water-soluble fractions the sugar content, estimated by the phenol–sulfuric acid method (Dubois et al., 1956), of *G. kola* is much lower than *C. acuminata* and *C. nitida* (Fig. 1A). Furthermore, for water-soluble fractions, a significant difference was detected in uronic acid content of *C. nitida*, *G. kola* and *C. acuminata* (Fig. 1B). The lowest of total sugar content in *G. kola* water-soluble fraction suggested that this fraction is enriched in water-soluble proteins or in glycoproteins such as AGPs. The total sugar content of CWM of *C. acuminata*, *C. nitida* and *G. kola* showed no difference between these species (Fig. 1B). However, for total uronic acid content, CWM of *G. kola* showed a much higher content than *C. acuminata* and *C. nitida* (Fig. 1B).

3.2. Monosaccharides composition

The total sugar composition of CWM of *C. acuminata*, *C. nitida* and *G. kola* was determined after trifluoroacetic acid (TFA) hydrolysis. This analysis, which excluded the TFA-resistant α-crystalline

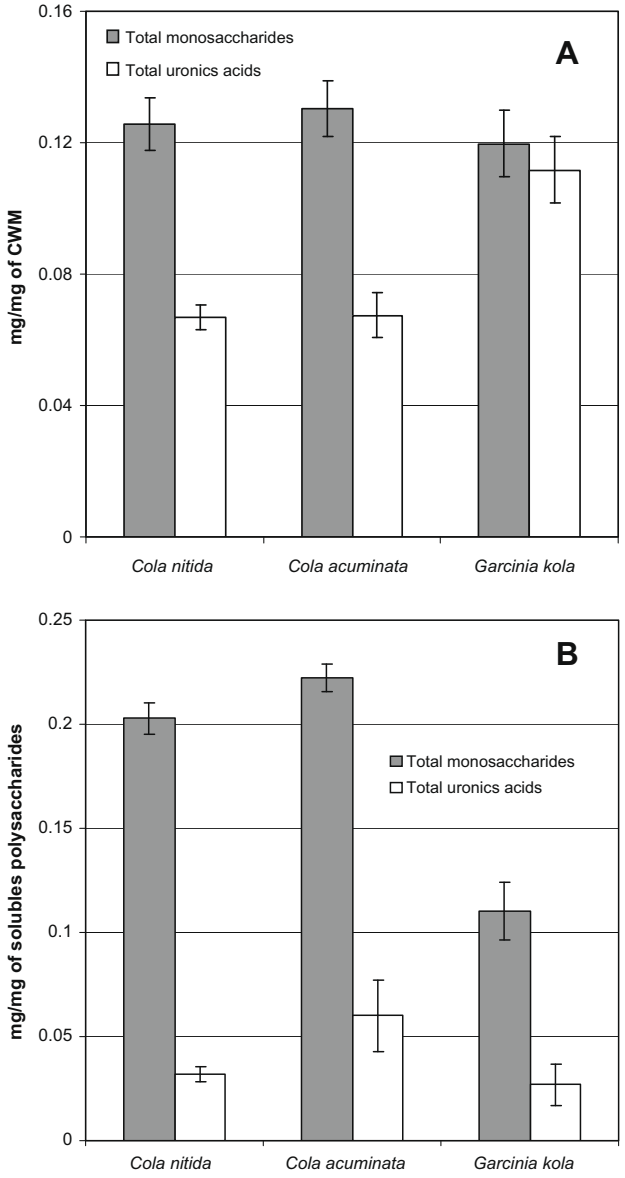

Fig. 1. Total sugar content and total uronic acid of CWM (A) and water-soluble polysaccharides (B) of *C. acuminata*, *C. nitida* and *G. kola* estimated by the phenol–sulfuric acid method (Dubois et al., 1956) and by Blumenkrantz and Asboe-Hansen (1973) method, respectively.

cellulose, revealed Gal (up to 30%), Ara, GalA and Glc as the predominant monosaccharides, representing approximately 90% of the total hydrolysable sugar present in this material (Fig. 2A). The other monosaccharides, present in decreasing order of concentration, were found to be Xyl, GlcA, Rha, Man and Fuc.

The monosaccharide composition of each fraction obtained after cell wall fractionation was determined (Fig. 2B–E). Ammonium oxalate fraction (Fig. 2B) contained predominantly, GalA, Gal, Glc and Ara. When omitted Glc, the presence of GalA (up to 30%), Ara and Gal was consistent with soluble pectin, specifically the homogalacturonans polymers. There were significant differences in the levels of Ara and Gal between these species. *G. kola* was found to contain high levels of Ara when *C. nitida* and *C. acuminata* showed a high level in GalA (Fig. 2B). Soluble hemicellulosic fractions (SF) contained predominantly Glc and Gal (Fig. 2C). There were significant differences in the level of Glc, Gal, Xyl, Ara and Man. The level of Glc was high in *C. acuminata* and *C. nitida*. The level of Gal and Xyl were high in *C. nitida* and *G. cola*. The level of GlcA and Man was high only in *C. acuminata*. Of these monosaccharides, increased amounts of and Glc and a decreased amount of GalA were found to be consistent with KOH-mediated solubilization of hemicellulose. Insoluble hemicellulose fractions (InF) contained Glc and Gal as the most abundant monosaccharides present, with increased levels of Gal found in *C nitida*. Glc was elevated in *C. nitida and in C. acuminata* (Fig. 2D). Cellulosic fractions (Fig. 2E) contained mostly Glc, Gal and Man. Glc most likely derived from crystalline cellulose. Furthermore, mannans have been reported to be associated with cellulose (Fry, 1988); the presence of Man in this fraction suggested such an association in *C. acuminata*, *C. nitida* and *G. kola* seeds. Extensive work is needed to elucidate the structure of pectins, hemicellulose and cellulosic fractions of CWM of *C. nitida*, *C. acuminata* and *G. kola*.

3.3. Analysis of arabinogalactan-proteins (AGPs)

Total AGPs extracted were estimated by rocket electrophoresis on agarose gel (Girault et al., 2000) (Fig. 3). Quantification of AGPs by rocket electrophoresis showed that *G. kola* seeds contained approximately 200.7 μg/g of Fw. While, *C. acuminata* and *C. nitida* contained approximately 50.02 and 25.3 μg/g of Fw respectively. Mono-dimensional electrophoreses of these molecules in agarose gels followed by staining with β-glucosyl Yariv reagent: one broad band was detected in all extracts with low mobility than that of our reference, the AGP from gum arabic (Fig. 4). After two-dimensional electrophoresis, one peak of AGPs was identified in *C. nitida*, while in *C. acuminata* we noticed three peaks (Fig. 5A–C). These three peaks characterized the diversity of AGPs families in *C. acuminata*. Although, *G. kola* showed a high level of AGPs, only one peak was identified in this fraction. The AGPs are often more heterogeneous in charge and in size due to different amounts of uronic acids and also of acidic amino acids (Girault et al., 2000). The monosaccharide composition of purified AGPs (Fig. 6) showed that the extracts of the seeds of the three plants contained predominantly Gal and Ara which are the main sugars of AGPs (Showalter, 2001). Other sugars, including Fuc, Xyl, Rha, Man and GalA were also present. This figure showed that AGPs from *G. kola* contained slightly more GlcA and GalA than those of *C. nitida* and *C. acuminata*, this difference being probably partly responsible for its higher mobility on agarose gel electrophoresis. Fig. 6 also showed that AGP from *G. kola* contained more Ara (36.8 ± 2.8%) than the others species. It is well known that the quality of gum depends on environmental factors, plant species, monosaccharides composition and others uncontrollable factors (Idris, Williams, & Phillips, 1998; Yadav, Igartuburu, Youchun Yan, & Nothnagel, 2007). Further investigations need to be carried out in order to check if the properties of the AGPs of the seeds of these three species could be related to their monosaccharides composition.

3.4. Phenols analysis

The total phenolic content was 49.36 ± 2.75; 31.6 ± 4.83 and 17.03 ± 3.03, respectively in *C. nitida*, *C. acuminata* and *G. kola*. Qualitative analysis of phenolics in *C. acuminata*, *C. nitida*, and *G. kola* after analylis by HPLC using a Waters 600E HPLC (Waters, Milford, MA) showed their diversity (Fig. 7). In *C. acumina* and *C. nitida*, they were largely represented by theobromine, catechin, epicatechin, and caffeine. Caffeine was the major compound and represented 48.93 ± 2.5% and 51.18 ± 2.2% of total soluble phenols of the seeds of *C. acuminata* and *C. nitida*, respectively (Fig. 7). Furthermore, catechin was the dominant flavonoid and it represented 34.45 ± 1.6% and 33.29 ± 2.2% of soluble phenolics of *C. acuminata* and *C. nitida* respectively (Fig. 7). The results obtained introduced

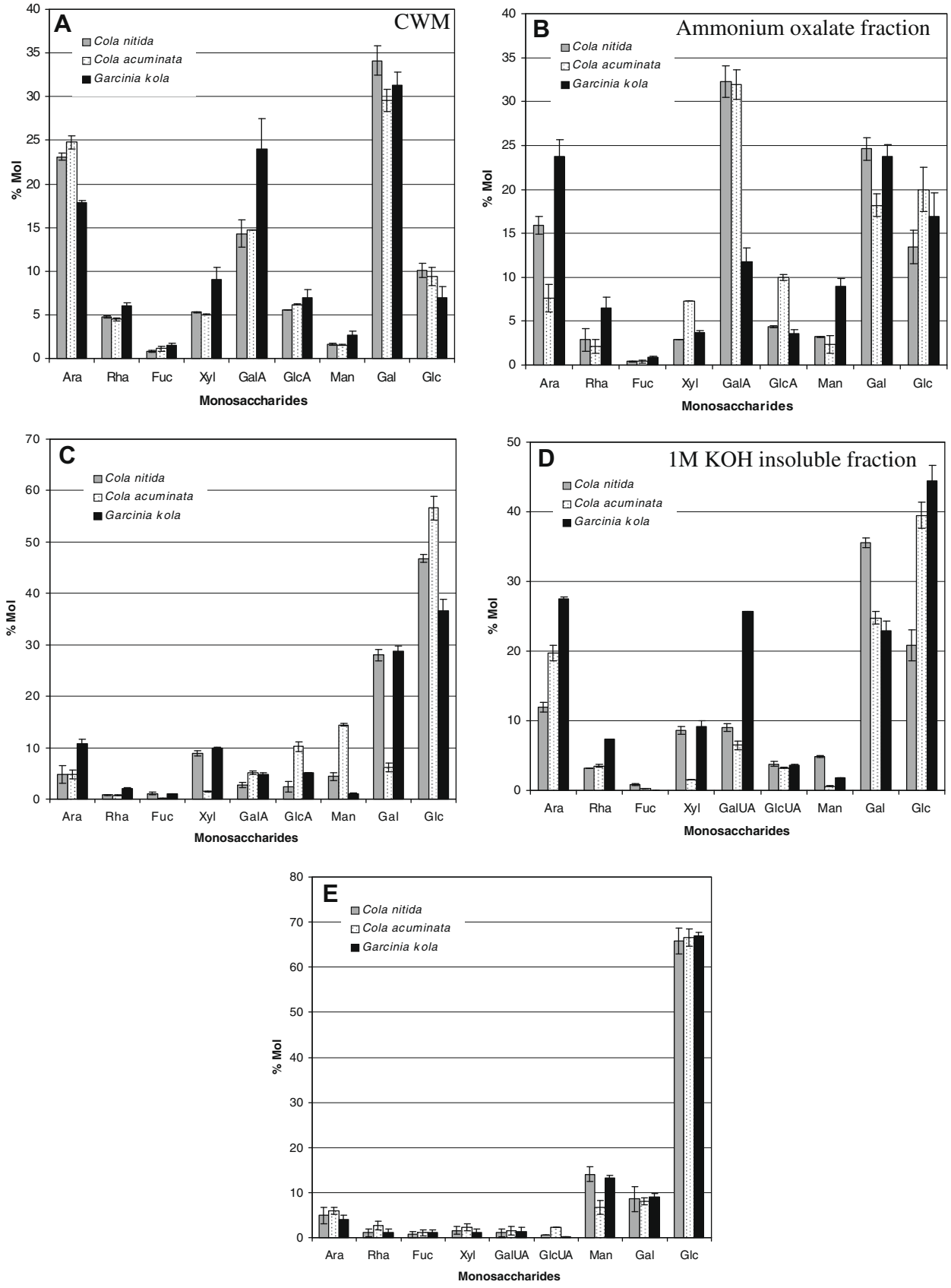

Fig. 2. Monosaccharide molar composition of polysaccharides fractions prepared from *Cola acuminata*, *Cola nitida* and *Garcinia kola*. (A) CWM (TFA hydrolysates). (B) Ammonium oxalate fraction. (C) 1 M KOH soluble fraction (KOH1A). (D) 1 M KOH insoluble fraction (KOH1B), (E) Cellulosic fraction (H_2SO_4 hydrolysate).

C. acuminata and *C. nitida* as a possible source of caffeine and catechin (Ashihara & Crozier, 1999; Lakenbrink, Lapczynski, Maiwald, & Engelhardt 2000; Peterson et al. 2005). In *G. kola*, with methanol or with acetone extraction. We detected four peaks

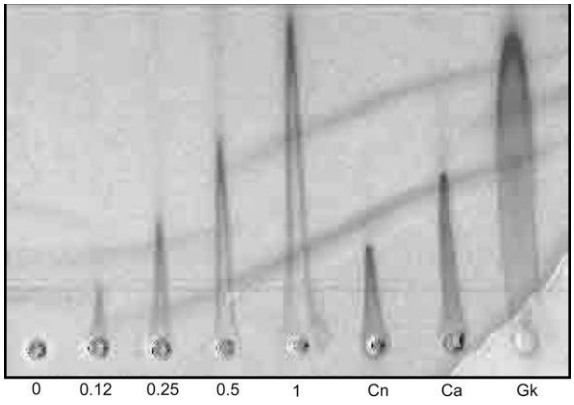

Fig. 3. Quantification of total purified arabinogalactan-proteins from *C. nitida*, *C. acuminata* and *Garcinia kola* by rocket electrophoresis. Arabic gum AGP are used as standard. (AG: Arabic gum; Cn: *Cola ninida*; Ca: *Cola acuminata*; Gk: *Garcinia kola*).

(Fig. 8) and not three peaks as previously described by Farombi (2000) following extraction with acetone. These compounds were probably biflavanones including GB-I-glucoside, GB-1, GB-2, and kolaflavonone. However, Highspeed-Counter-Current Chromatography (HSCCC) was recently used to identified five biflavanones in seeds of *G. kola* brought from a local market at Orba Nsukka, Enugu State, Nigeria (Okunji et al., 2007). This difference could be attributed to the geographical origin of seeds.

4. Conclusion

After analysis, the three kola species studied are quite different from each other. Their water-soluble polysaccharides and cell wall polysaccharides are similar, but demonstrate important differences

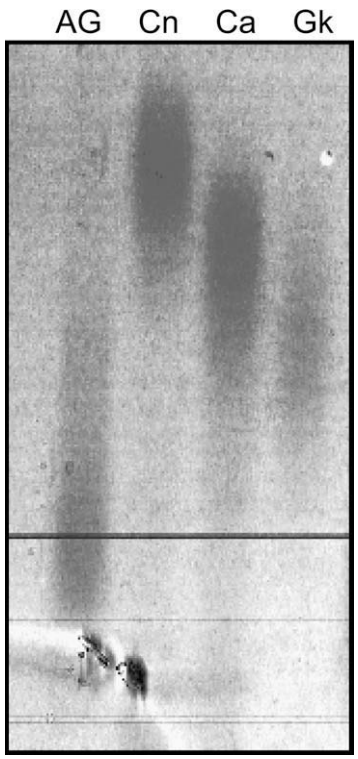

Fig. 4. Agarose gel electrophoresis of purified AGPs from *C. nitida*, *C. acuminata* and *G. kola*. Mono-dimensional electrophoreses; Arabic gum AGPs are used as standard. (AG: Arabic gum; Cn: *Cola ninida*; Ca: *Cola acuminata*; Gk: *Garcinia kola*).

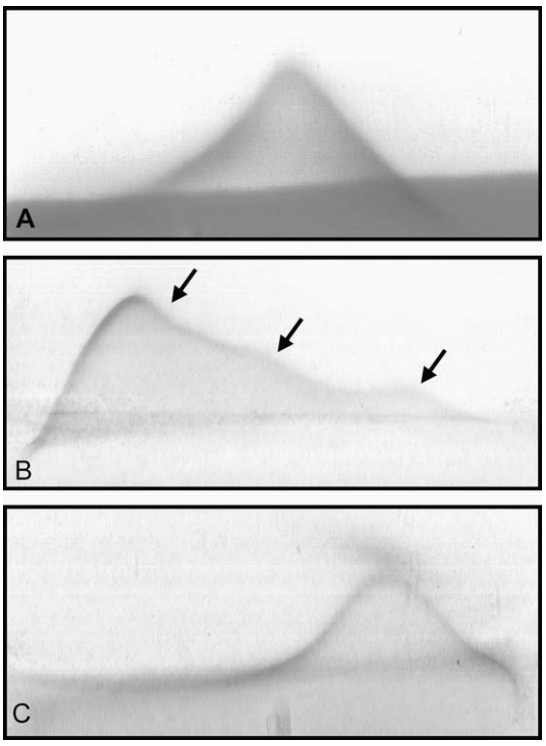

Fig. 5. Two-dimensional electrophoresis; (A) *C. nitida*; (B) *C. acuminata* and (C) *G. kola*.

on the basis of their percentages of polysaccharides per g of Fw, their monosaccharides composition and their phenolics (quantity and quality) contents. The total sugar composition of CWM of *C. acuminata*, *C. nitida* and *G. kola* showed that only Ara, and GalA displayed some significant differences between the three kola with a high quantity of Ara in both *C. acuminata* and *C. nitida* and an a high level of GalA in *G. kola* fractions. In hemicellulosic fractions, the levels of Ara and GalA were high in *C. acuminata* and *G. kola*, and that of Man was elevated in *C. nitida*. The Xyl level was high in *C. nitida* and *G. kola*. Quantification of AGPs by rocket electrophoresis showed that *G. kola* seeds contained approximately

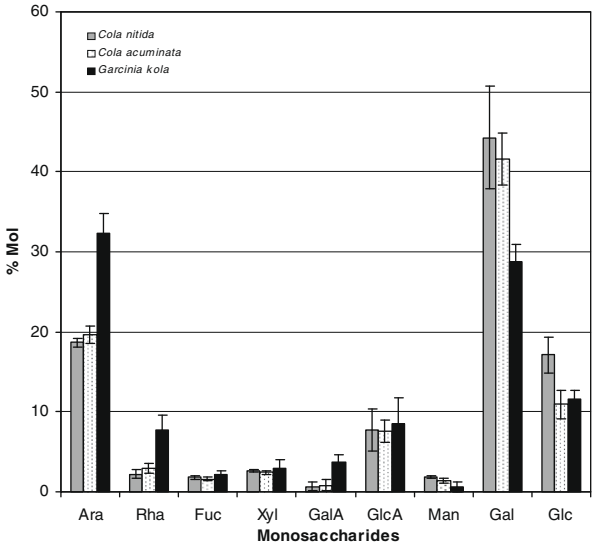

Fig. 6. Monosaccharide composition (mol%) of purified fraction of AGPs (B) extracted from *C. nitida*, *C. acuminata*, *G. kola seeds*.

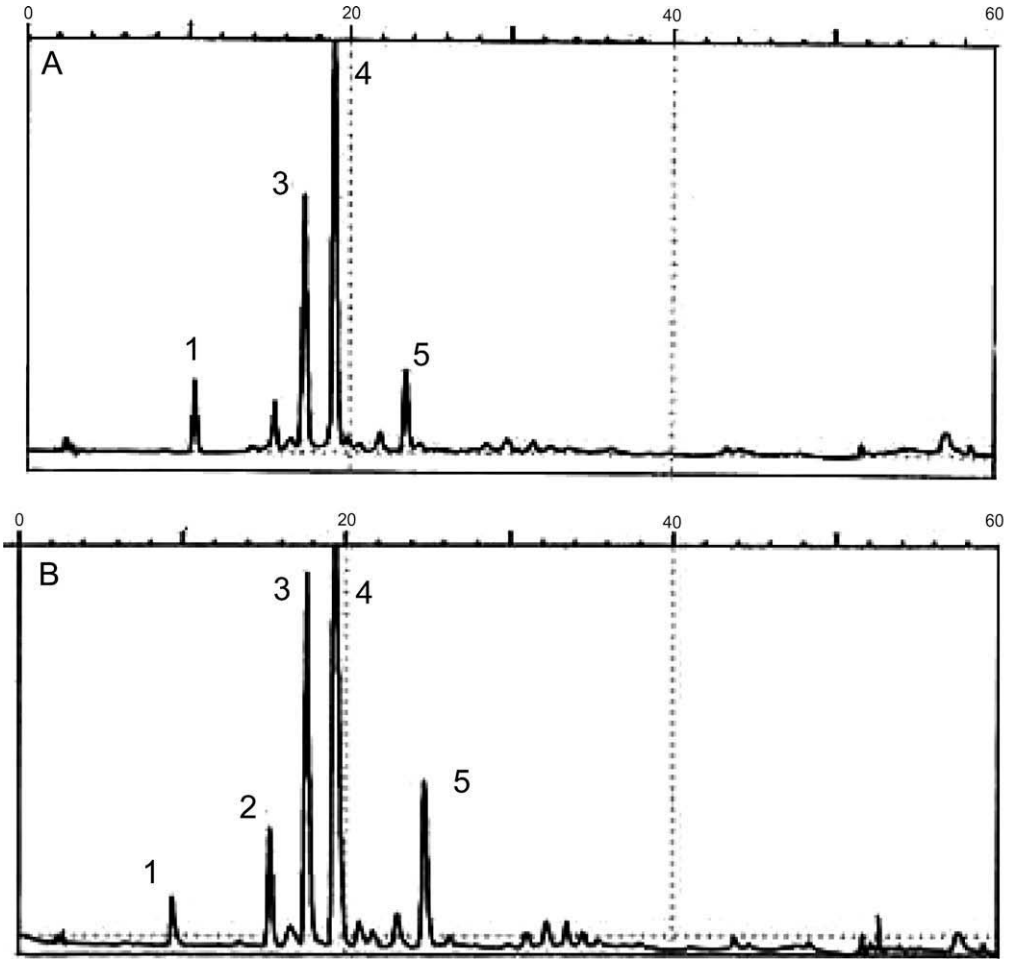

Fig. 7. HPLC chromatogram at 280 nm of phenolic compounds in *C. nitida* (A) and *C. acuminata* (B).

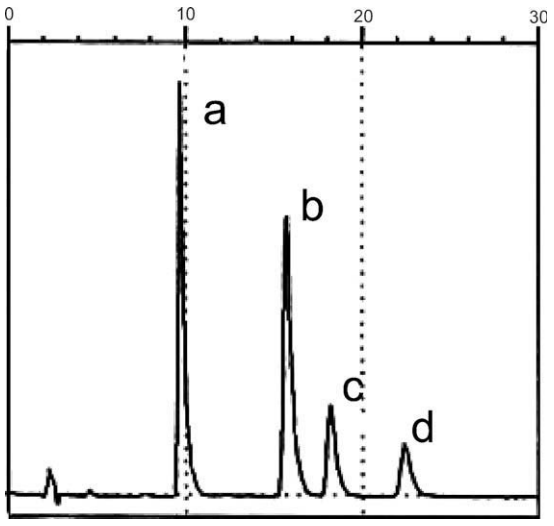

Fig. 8. HPLC chromatogram at 280 nm of phenolic compounds in *G. kola*.

200.7 μg/g of Fw. While *C. acuminata* and *C. nitida* contained approximately 50.02 and 25.3 μg/g of Fw respectively. After two-dimensional electrophoresis, one peak of AGPs was identified in *C. nitida* and *G. kola*, while in *C. acuminata* we noticed two peaks. Qualitative analysis of phenolics in *C. acuminata*, *C. nitida*, and *G. kola* showed the diversity of the compounds present after being analysis by HPLC. In *C. acumina* and *C. nitida*, they were largely represented by caffeine and catechin. Caffeine was the major compound and represented ~50% of soluble phenolics in these seeds. The present study provided information which could serve to explain some biochemical activities of the cola and their possible utilisation in the development as a functional food.

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