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Full Length Research Paper

Optimization of water-extract of phenolic and antioxidant compounds from Kinkéliba (*Combretum micranthum*) leaves

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The hot water extract of dry kinkéliba leaves (DKL) is a traditionally widely delighted and consumed beverage in West Africa, especially during the fasting periods. It is believed to possess various health benefit effects such as diuretic, purgative, digestive, anti-viral and anti-malarial properties. However, the preparation of this beverage has not been optimized so far, which may underestimate its health benefit potential. In this study, the traditional preparation of the beverage, by decoction, was optimized in order to maximize the amounts of health benefit polyphenolic and other antioxidant compounds. The results showed that an optimal preparation with maximum solubilization of polyphenols required the brewing of approximately 20-25 g of DKL in boiling water for about 15-20 min. These conditions afforded an optimized extract (OEDKL) with a total phenolic content of 215.0 mg GAE/g and arabinogalactan-rich pectic polysaccharide content of 553.0 mg/g. Moreover, the OEDKL exhibited a high antioxidant capacity, as evaluated by DPPH [93.0% IP and EC_{50} = 15.0 (g/g)], ABTS (112.0 µmol TE/ g), or FRAP (376.0 µmol TE/g) assay, which was correlated with the total phenolic content (R^2 = 0.897-0.991). Nevertheless, the pectic material detected within the beverage was likely to contribute to its interesting antioxidant properties. Therefore, it was suggested that the total antioxidant capacity of the OEDKL could result from synergistic interactions among the various antioxidant compounds present, including polyphenols and fractionated pectic materials.

Key words: *Combretum micranthum*, Beverages, Polyphenols, Pectic polysaccharides, Antioxidant properties.

INTRODUCTION

Free radicals, of which reactive oxygen species (ROS) are the most threatening, play a crucial role in the pathogenesis of various human disorders, such as colonic cancer, rheumatoid arthritis, neurodegenerative, coronary-heart, and pulmonary diseases (Soobrattee et al., 2005). In vivo, some of the free radicals play positive roles in cell physiology; however, the balance between the production of ROS and their neutralization by radical scavenging antioxidants is very delicate, and if this balance turns to overproduction of ROS relative to the antioxidant stores, normal cells start to suffer the consequences of oxidative stress and related disorders (Carocho and Ferreira, 2013).

Phenolic compounds are usually associated with the health benefits, which stem from the consumption of polyphenol-rich food stuffs such as fruits, vegetables, teas, coffees, and cocoa-derived products (Balasundram et al., 2006; Martin et al., 2013). A direct relationship between the total antioxidant activity (TAOC) and total phenolic content (TPC) of diverse (food) plant materials has widely been reported (Soobrattee et al., 2005; Carocho and Ferreira, 2013). Epidemiological studies have also shown that intake of polyphenols can effectively reduce the risk of development of various free radical-induced pathologies (Maskarinec, 2009). Besides,

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it has recently been reported that biologically active pectin-derived polysaccharides from various plant fruits,stems, roots, and leaves, with commonly known immunological and anti-tumor properties (Inngjerdingen et al., 2005; Yapo, 2011), may also be endowed with antioxidant properties (Gan and Latiff, 2011). Thus, there is a growing interest for seeking for food herbals and plants with high amounts of natural antioxidant compounds (AOC) to compensate for their shortage in refined modern diets. A diet with low AOC is indeed considered a risk factor for the development various free radical-induced disorders (Ching et al., 2006). The intake of AOC-rich foodstuffs are therefore of primary importance for maintaining optimum health balance and welfare.

Kinkéliba (*Combretum micranthum* G. Don), also known as kinkiliba (or quinquéliba or quinquiliba), is a dicotyledonous plant which belongs to the family of Combretaceae. It is generally an undomesticated shrub (4-5 m height) species which develops in the savannah regions of most West African countries (Burkina Faso, Côte d'Ivoire, Guinea, Mali, Niger, and Senegal). The fresh leaves are usually used to treat malarial fever (Benoit et al., 1996; Welch, 2010). The water extract of the leaf, the most biologically active plant tissue relative to stem, bark and roots, is one the most popular beverages in West Africa, especially in Senegal (Welch, 2010). The dark green oval leaves are usually harvested and sundried, before being packaged and sold as dried kinkeliba leaves (DKL) in local stores for tisane preparation, most often with a specified direction for use. Moreover, the health benefits, especially the pharmacological properties, of kinkéliba leaves are being recognized worldwide (Welch, 2010). The beverage is commonly prepared by decoction by brewing DKL in boiling water. In traditional preparation of the so-called "long life tisane" of DKL, as recommended by producers and sellers in Côte d'Ivoire, 20 g of DLK are usually boiled in 1.5 L of water for 3-5 min, cooled to room temperature and filtered, after which the filtrate is consumed as a hot beverage or a cold drink within 24 h after being cooled to 5-7 °C. The beverage has good taste and is nearly substituted for commercially available teas. In addition, it may possess various health benefit properties, including diuretic, purgative, digestive, anti-inflammatory, anti-viral, anti-nausea and anti-malarial potentials (Ferrea et al., 1993; Benoit et al., 1996; Olajide et al., 2003; Ancolio et al., 2002; Welch, 2010). It is may also be effective against spleen fever and liver disorders, and Type 2 diabetes (Welch, 2010). These functional properties are believed to stem from various biologically active components present within DKL, namely high amounts of alkaloids, betains, heterosides, polyphenols, tannins, and potassium nitrate (Ferrea et al., 1993; Karou et al., 2005; Welch, 2010; Udoh et al., 2012). However, all these previous studies have used either (aqueous) organic solvent (acetone, alcohols, etc.) extractions or a one-point pure water extraction without any optimization of the extraction process. On the one hand, the use of aqueous-organic solvents, as commonly done, in the evaluation of TPC and TAOC of plant extracts does not reflect the traditional preparation of this beverage by decoction. On the other hand, the utilization of a one-point pure water extraction, without any optimization may solubilize low quantities of these bioactive compounds. The scope of this study was then to optimize the traditional preparation of DKL beverage in order to enhance its TPC and TAOC.

MATERIAL AND METHODS

Reagents, Enzyme Solutions, and Analytical Apparatus

2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-Tetramethylchromane-2-carboxylic acid (Trolox, a water-soluble vitamin E analogue), Folin-Ciocalteu reagent (FCR), Gallic acid, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), and Glucose oxidase (GOD; EC 1.1.3.4) were from Sigma-Aldrich Co. (St. Louis, MO).

All the UV-Vis spectrophotometric analyses were performed with a Perkin-Elmer Lambda 3 Double Beam Spectrophotometer (PerkinElmer Corp., Norwalk, CT).

RAW PLANT MATERIALS

Dry kinkéliba (*C. micranthum* G. Don) leaves (DKL) were purchased from a local distributor of African medicinal plant products (CIPRADEL, Abidjan, Côte d'Ivoire). The plant materials were authenticated at the department of Botany, University of Felix Houphouet Boigny (ex. University of Abidjan-Cocody).

Optimization of the Water Extraction of Bioactive Compounds from DKL

In a first experimental procedure, DKL (20 g) were treated in boiling water (1.5 L) for 3-60 min, cooled to room temperature, and filtered through G-3 sintered glass, and then on nylon cloth (0.45 µm). The different filtrates were concentrated, by rotary evaporation (40 °C), to the desired quantity of solutions, freeze-dried, and kept at room temperature under moisture-free conditions pending analysis. In a second experimental procedure, 5-55 g of DKL were treated in 1.5 L boiling water for 15 min and processed as above to obtain filtrates, which were concentrated to the desired quantity of solutions, freeze-dried, and stored under moisture-free conditions until analysis. According to the traditional preparation, as

specified by producers and sellers, 20 g of DKL are boiled in 1.5 L of water for 5 min, cooled to room temperature and filtered and processed as above. The product obtained was referred to as the control extract. All the experiments were performed in three independent runs.

Determination of the total phenolic content of DKL

The total phenolic content (TPC) of the extracts was determined by a colorimetric FCR assay (Singleton et al., 1999). Briefly, samples (0.1 mL) were added to test tubes, followed by addition of 6 mL of distilled water and 0.5 mL of FCR. After mixing and resting for 3 min, 1.5 mL of 20% (w/v) sodium carbonate were added to test tubes and adjusted to 10 mL. The mixtures were thoroughly vortex-mixed, and the increase in absorbance of the colour generated, after 2 h at room temperature, was spectrophotometrically read at 760 nm against an appropriate control (or "blank"). Gallic acid (25-500 µM) was used as standard and the results obtained were expressed as gallic acid equivalents (mg GAE)/g dried weight of sample. Analyses were carried out in triplicates.

Determination of the Total Antioxidant Capacity (TAOC)

DPPH Scavenging Antioxidant Assay

The total antioxidant capacity (TAOC) of the extracts was first evaluated for its ability to scavenge the DPPH free radical by two ways.

For the fixed duration reaction method (Mishra et al., 2012), 0.2 mL ethanolic solution of antioxidant-containing sample (0.5 mg/mL) was added to 0.8 mL of a 50 mM Tris-HCl buffer (pH 7.5) and vortex-mixed, followed by addition of 1 mL of a 0.1 mM ethanolic solution of $DPPH^{\bullet}$. The reaction mixture was thoroughly vortex-mixed and left in the dark at 23 °C for 30 min. The decrease in absorbance of the mixture, at room temperature, was spectrophotometrically measured at 515 nm. Trolox (0.1-1.0 mM) was used as antioxidant standard. Antioxidant activity or inhibition percentage (IP), defined as the ability of the sample to scavenge the DPPH free radical, was calculated as follows:

$IP\ (\%) = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

where $Abs_{control}$ and Abs_{sample} are the absorbances of the free radical $DPPH^{\bullet}$ in the absence and presence of the antioxidant-containing sample, respectively.

For the steady sate saturation method (Brand-Williams et al., 1995), 0.1 mL of an ethanolic solution of sample was added to 3.9 mL of a 24 mg/L of ethanolic solution of DPPH. The decrease in absorbance was determined at 515 nm at different time intervals until the reaction reached a steady state (a plateau-like curve). The initial concentration of $DPPH^{\bullet}$ in the reaction medium was calculated from a calibration curve, as obtained by linear regression ($R^2 = 0.989$), and the percentage of remaining $DPPH^{\bullet}$ at steady state ($\%DPPH^{\bullet}_{Rem}$) was calculated and plotted against the sample concentration. The antiradical activity was determined in terms of the efficient (or inhibition) concentration (EC_{50} (g dry weight sample/g $DPPH^{\bullet}$)), viz. the amount of antioxidant-containing sample required for 50% decrease in the initial $DPPH^{\bullet}$ concentration.

ABTS scavenging Antioxidant Assay

The TAOC of the extracts was also evaluated by its capability to scavenge ABTS radical (Re et al., 1999). Briefly, 7 mM ABTS stock solution was prepared in water. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting equal quantities of ABTS stock solution with 2.5 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 15 h before use. The generated $ABTS^{\bullet+}$ solution (1 mL) was then diluted with ethanol (60 mL) to an initial absorbance of 0.802 ± 0.01, read at 734 nm, and the reagent obtained was equilibrated at 30 °C. After adding 0.9 mL of the latter $ABTS^{\bullet+}$ solution to 0.1 mL of antioxidant-containing sample in test tube and vortex-mixing, the decrease in absorbance of the mixture, at 30 °C, was spectrophometrically measured at 734 nm for 1-7 min against an appropriate control (or "blank"). Trolox (0.1-1.0 mM) was used as standard and the results were expressed as micromole of Trolox equivalent (µmol TE)/g dried weight of sample. Analyses were carried out in triplicates for each sample used.

Ferric Reducing Antioxidant Power (FRAP) Assay

The TAOC of the extracts was finally evaluated through its ability to reduce ferric (Fe^{3+}) cation to ferrous (Fe^{2+}) cation (Benzie and Strain, 1996). The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working FRAP reagent was prepared by mixing acetate buffer, TPTZ, and $FeCl_3.6H_2O$ solutions in a ratio of 10:1:1 (v/v/v), respectively; and was warmed up to 37 °C before use. Antioxidant-containing sample (0.1 mL) was added to 0.9 mL of the FRAP solution, and the mixture was vortex-mixed and allowed to react for 30 min in the dark. The increase in absorbance of the mixture, at 37 °C, was spectrophotometrically monitored at 593 nm for 4-30 min against an appropriate control (or "blank"). Trolox (0.1-1.0 mM) was used as standard and the results were expressed as µmol of TE/g dried weight of sample.

Sugar Composition Analyses

Aliquots were taken from the extracts with the highest

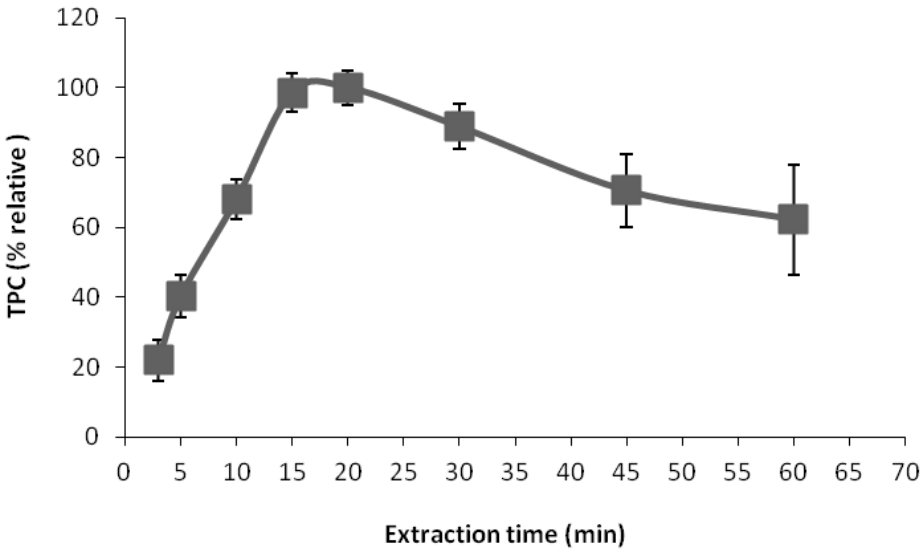

Figure 1. Effect of extraction time on the total phenolic content (TPC) of hot water-solubilized antioxidant fractions from dry kinkeliba leaves.

TAOC and mixed with 3 volumes of 70% (v/v) aqueous ethanol. The mixtures were then allowed to rest at 5 °C overnight and centrifuged (3000*g*, 20 min) to recover precipitates, which were dispersed in distilled water and extensively dialyzed in 3500 molecular weight cut off tubing against distilled water. The retentates were concentrated by rotary evaporation (40 °C) to desired quantity of solutions and were analyzed for glycosyl residue composition. They were therefore treated with 1 mol.L^{-1} H_2SO_4 (100 °C, 3 h) to release the monosaccharide constituents of the solubilized polysaccharide material as described previously (Yapo and Koffi, 2008). Galactose, glucose, mannose, and fucose were analyzed after Finch et al. (1969). Glucose was also analyzed by the rapid GOD-Fenton's reaction method (Woodward et al., 1985). Arabinose and rhamnose were determined using Megazyme assay kits (Megazyme International Ireland Ldt., Bray, Co. Wicklow, Ireland). Xylose was analyzed according to Pham et al. (2011) and finally uronic acid was determined as galacturonic acid as previously published (Yapo, 2010). All the analyses were carried out in triplicates for each sample used.

Statistical Analysis

All the data were statistically appraised by a single-factor analysis of variance (ANOVA), followed by the Bonferroni's posthoc test for multiple comparisons, whenever applicable, using a GraphPad Prism V.3 software (GraphPad software Inc., San Diego, CA). The means of different treatments were considered to be significantly different at P-value <0.05. Correlation between the TPC and TAOC of extracts were determined by the Pearson's test.

RESULTS AND DISCUSSION

Optimization of the Water Extraction of Total Phenolic Compounds

The result of effect of the duration of extraction on the amount of water-solubilized phenolics from DKL is shown in Figure 1. As can be seen, the TPC of the beverage increased with increasing time of extraction up to 20 min, after which it decreased substantially as the extraction time was further increased to 60 min. Longer heating times have also been found to decrease the TPC and TAOC of soluble phenolics from grape seed flour (Ross et al., 2011). These results indicated that the heating duration considerably influenced the TPC of the beverage, thereby showing the importance of the optimization of the extraction process. The highest TPC was obtained after 15-20 min extraction. In comparison with the latter result, the TPC of the control extract was significantly lower (P <0.05), representing approximately 20-40% of the maximum yield obtained at 15-20 min extraction. This confirmed that a longer time of extraction (than 5 min) was required for maximizing the solubilisation of phenolics from DKL.

Furthermore, the variation in the initial quantity of DKL used for the preparation of the beverage showed that the best yield of phenolics was obtained when 20-25 g of DKL were boiled in 1.5 L of water for 15 min (Figure 2). This solid to liquid ratio is in good accord with the one traditionally recommended. The optimized extract from DKL (OEDKL), obtained by boiling 20 g of DKL in 1.5 L

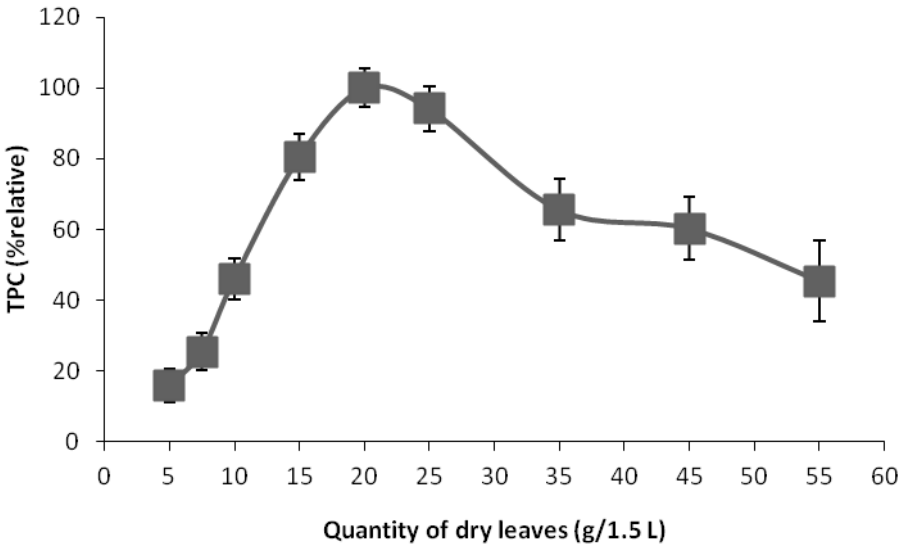

Figure 2. Effect of solid to liquid (S/L) ratio on the total phenolic content (TPC) of hot water-solubilized antioxidant fractions from dry kinkeliba leaves.

for 15 min and the control extract were further analyzed for TPC and TAOC.

Total Phenolic Content (TPC) and Antioxidant Capacity (TAOC) of Extracts

The results of the analysis of the TPC and TAOC of OEDKL and control extract are presented in Table 1. The TPC of OEDKL and control extract were 214.8 and 79.6 mg GAE/g, respectively; showing that there were 2.7 times more phenolics in OEDKL than in the control extract. The TPC of OEDKL was lower than the one (*ca.* 378 mg GAE/g) reported by Karou et al. (2005). This difference is likely due to difference in the methods used for the extraction of polyphenols from kinkéliba leaves, 70% (v/v) aqueous acetone being used, as commonly done, by the latter workers. 60-70% aqueous organic solvent (acetone and/or alcohol) extractions are known to be more efficient that polar solvent (water) extraction (Cheynier et al., 1999). However, it should be underlined that we performed only pure water extraction, and not aqueous organic-solvent extraction, to be in line with the traditional preparation of the beverage from DKL. The differing origin of kinkéliba leaves (Burkina Faso in their study) may also contribute to discrepancies in data to some extent. However, the TPC of OEDKL was higher than those reported in other hot beverages such as instant and ground coffees (52.5-151.0 mg/g) and black and green teas (61.0-200.0 mg GAE/g) as summarized elsewhere (Balasundram et al., 2006).

The TAOC of OEDKL and control extract was evaluated by DPPH, ABTS, and FRAP methods, because the TAOC of a (food) plant material generally results from a mixture of various antioxidant compounds with different mechanisms of action, among which there may be synergistic (or antagonistic) interactions (Aruoma, 2003; Pérez-Jiménez et al., 2008). Moreover, there is not one analytical method that can provide unequivocal results (Carocho and Ferreira, 2013). Therefore, a better practice nowadays recommends the combination of (at least two) different methods for the in vitro determination of the TAOC of food products. The DPPH radical measures a sample's free radical scavenging capacity, which is based on the premise that a single electron transfer (SET) or a hydrogen donor is an antioxidant. The ABTS radical also measures a sample's free radical scavenging capacity. It is commonly referred to as a decolorization assay in which there is only a SET reaction. FRAP measures the ability of a sample to reduce metal cations and is also based on a SET reaction (Pérez-Jiménez et al., 2008; Carocho and Ferreira, 2013).

The TAOC of OEDKL, as determined by the colorimetric DPPH, ABTS, and FRAP assays, were 92.8% IP of DPPH (EC50 = 14.5 g/g), 112.4 µmol TE/g, and 375.9 µmol TE/g, respectively. The results of the TAOC of control extract, as determined by DPPH, ABTS, and FRAP assays, were 52.6% IP of DPPH (EC_{50} = 31.2 g/g), 49.7 µmol TE/g, and 104.8 µmol TE/g, respectively (Table 1). It can be seen, in each case, that the TAOC of OEDKL was significantly greater than that of the control extract ($P < 0.05$), thereby demonstrating the necessity for the optimization of the preparation of this beverage.

In comparison with other workers, the TAOC of OEDKL, as appraised by the DPPH assay (EC_{50} = 14.5 g/g), is rather comparable to the one (9.1 ± 0.28) found for a water extract of kinkéliba leaves from Guinea (Touré

Table 1. Total phenolic content, total antioxidant capacity, and glycosyl residue composition of the optimized extract (OEDKL) and control extract from dry kinkeliba leaves.

Antioxidant properties	**OEDKL**	**Control extract**
Soluble phenolics (mg GAE/g)	214.8 ± 5.7a	79.6 ± 2.2b
DPPH (%IP)	92.8 ± 3.6a	52.6 ± 1.7b
DPPH [EC_{50} (g/g)]	14.5 ± 0.2a	31.2 ± 1.4b
ABTS (µmol TE/g)	112.4 ± 5.7a	49.7 ± 2.3b
FRAP (µmol TE/g)	375.9 ± 3.4a	104.8 ± 2.7b
Sugar composition (mol%)		
Rhamnose	14.8 ± 1.4	ND
Fucose	nd	ND
Arabinose	12.6 ± 1.1	ND
Xylose	3.2 ± 0.4	ND
Mannose	nd	ND
Galactose	34.7 ± 4.3	ND
Glucose	8.2 ± 0.9	ND
Galacturonic acid	26.4 ± 2.7	ND
Total sugar (mg/g)	553.1 ± 6.5	ND

Data are expressed on a dry weight basis as mean ± SD (*n* =3). Values in the same lines with different letters are significantly different (*P <0.05*).
nd: not detected
ND: not determined

et al., 2011). The TAOC of OEDKL, as evaluated by the ABTS assay, is much lower than that (16.37 µmol TE/µg) reported by Karou et al. (2005). This discrepancy might be explained by a lower efficient of pure water extraction, compared with 70% aqueous acetone extraction (in their study) which is known to solubilize high molecular weight polyphenols, especially proanthocyanidins (Cheynier et al., 1999). Touré et al. (2011) have also observed that the EC_{50} (or IC_{50}) values of the ethanol extract of Guinean kinkéliba leaves in ABTS and DPPH tests were significantly lower than those from the water extract. A strong correlation was found to exist between the TPC and TAOC of OEDKL for either FRAP (R^2 = 99.1%) or ABTS (R^2 = 97.8%). By contrast, the correlation was moderately high for DPPH (R^2 = 89.7%). Karou et al. (2005) have also found that the TPC of 70% aqueous acetone extract of kinkéliba leaves, from Burkina Faso, were highly correlated (r = 0.91) with the antioxidant activities using the ABTS assay.

In order to know if polysaccharide components were also present in the hot water extract (OEDKL), since biologically active (pectic) polysaccharides, which are generally present in all higher plant tissues, are usually extracted by this method (Inngjerdingen et al., 2005; Yapo, 2011), 70% aqueous alcohol-precipitation was carried out, thereby partitioning the hot water-extract into alcohol-soluble and alcohol-insoluble (precipitate) fractions. The latter fraction was then analyzed for different typical monosaccharide constituents of polysaccharides. The results are also shown in Table 1. The quantitatively detected monosaccharides, in order of abundance, were galactose (34.7 mol%), galacturonic acid (26.4 mol%), rhamnose (14.8 mol%), arabinose (12.6 mol%), glucose (8.2 mol%), and xylose (3.2 mol%). This indicated that polysaccharides (553.1 mg/g), mainly pectic substances with possibly some xyloglucan moieties, were co-extracted with polyphenols by the hot water treatment of DKL.

To substantiate the polysaccharide contribution or not to the TAOC of the overall water extract (OEDKL), the derived alcohol-soluble and alcohol-insoluble fractions were analyzed by DPPH, ABTS, and FRAP assays. It was observed that each of the two fractions exhibited substantially lower TAOC, compared with that of the whole extract (data not shown). It was therefore suggested that the interestingly high TAOC of OEDKL resulted from synergistic interactions among various biologically active compounds, including at least arabinogalactan-rich pectic polysaccharides and polyphenols, of which gallic acid might be the major components (Touré et al., 2011). This observation is in agreement with the recent findings that pectic polysaccharides from mangosteen (Gan and Latiff, 2011) are effective free radical (DPPH) scavengers. The free radical scavenging activity and metal-reducing power of pectic polysaccharides could be explained by the

presence of several protonated carboxyl (-COOH) groups and secondary alcohol (-OH) groups, which render them potential hydrogen donors and contributors to the single electron transfer mechanism. Thus, in addition to polyphenols, pectic polysaccharides may be the other effective antioxidant compounds present within the OEDKL.

CONCLUSION

Investigation on the bioactive compounds content of the beverage, obtained by decoction, from dry kinkeliba leaves showed the presence of high amounts of soluble phenolics, together with antioxidant pectic polysaccharides. The product obtained (OEDKL) under optimized conditions exhibited interesting antioxidant properties, which is likely to result from synergistic interactions among various compounds, including polyphenols and fractionated pectic materials. The wide interest for daily consumption of this beverage is therefore fully accounted for. However, as demonstrated in this study, the traditional preparation of the beverage should be optimized by brewing approximately 20-25 g of the dried raw material in 1.5 L of boiling water for about 15-20 min in order to enhance its health benefit potentials. Our future study should deal with identification of the specific classes of the antioxidant compounds present in OEDKL and in vivo analyses.

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A list of Abbreviations used

ABTS: 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonic acid)
AOC: Antioxidant compounds
DKL: Dry kinkeliba leaves
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
FCR: Folin-Ciocalteu reagent
FRAP: Ferric reducing antioxidant power
GAE: Gallic acid equivalents
GOD: Glucose oxidase
OEDKL: Optimized extract of dry kinkliba leaves
TAOC: Total antioxidant capacity
TPC: Total phenolic content
TPTZ: 2,4,6-Tri(2-pyridyl)-s-triazine

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