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Polyphenols content and antioxidant capacity of traditional juices consumed in Côte d'Ivoire

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ABSTRACT

Objective: This study aims at determining the total phenol content and antioxidant potency of traditional juices consumed as soft beverages in Côte d'Ivoire.

Methodology and Results: The total polyphenol content (TPC) of juices of baobab fruit pulp, passion fruit pulp, lemon, tamarind pulp, also Roselle calices and ginger roots was determined by the Folin-Ciocalteu (FC). Their antioxidant capacity was assessed as ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and the radical-cation $ABTS^{\bullet+}$, and the ferric reducing antioxidant power (FRAP). An overall antioxidant composite index (ACI) of each juice was determined by calculating an index score referring to an index value of 100, assigned to the best score of antioxidant capacity for each test and the index value of the juice. TPC ranged from 3.7 ± 2.49 mg of gallic acid equivalents/mL of juice for lemon to 50.1 ± 15.5 mg of gallic acid equivalents/mL of juice for baobab. The rank order of TPC of the investigated juices was baobab > Roselle > tamarind > ginger > passion > lemon. Baobab fruit juice and Roselle calices juices exhibited the highest antioxidant potency with the three DPPH, FRAP and ABTS methods and had the highest antioxidant composite index (89.5 and 96.1 respectively). These juices are followed by tamarind fruit juice with values of TPC and ACI equal to 22.92 mg of gallic acid equivalents/mL of juice and 74.4 respectively.

Conclusions and application of findings: This study shows that baobab juice contains the highest total polyphenol amount, followed by Roselle juice. Although all the investigated juices exhibit an antioxidant activity, these two beverages present the highest antioxidant capacities. Regarding these findings, Roselle baobab and Tamarind juice juices are the most promising antioxidant sources and should be promoted as functional beverages to fight against chronic diseases in Côte d'Ivoire.

Keywords: juices, homemade, total phenol, antioxidant activity, Côte d'Ivoire

INTRODUCTION

Fruits juices of baobab (*Adansonia Digitata* L.) passion (*Passiflora edulis* F.), lemon (*Citrus limon*), tamarind (*Tamarindus indica* L), also Roselle (*Hibiscus sabdariffa*) calices and ginger (*Zingiber officinale* (L) Rose) roots are consumed as beverages in Côte d'Ivoire. They are processed into juice by women at home level and chilled for sale. Thus, they are consumed as ice drinks mainly in urban areas. It is found in literature that all plants involved in the present study, are valuable source of

polyphenols, which exhibit antioxidant activity against free radicals caused by oxidation of polyunsatured fatty acids in human body cells membranes. (Rice-Evans & Miller, 1996; Salah *et al*. 1995, Proteggente *et al*., 2002, Tsai *et al*., 2002, Lamien-Meda *et al*., 2008, Shirin & Jamuna, 2010, Yariwake et *al*., 2010; Brady, 2011; Hajimahmoodi *et al*. 2012, Rekha *et al*., 2012, Yang *et al*., 2012). These free radicals, which are chemical compounds, induce serious damage such as coronary atherosclerosis, emphysemas, cancer and cirrhosis (Stoilova *et al*., 2007). To this regards, the baobab fruit pulp traditionally used in Africa to prepare decoctions and natural refreshing drink, was reported to have a higher antioxidant capacity than orange, kiwi, apple and strawberry pulps, commonly considered rich in antioxidants (Vertuani *et al*., 2002, Besco *et al*., 2007). Some studies have shown that ginger roots and resulting extracts contain polyphenolic compounds (6-gingerol and derivatives), which have a high antioxidant activity (Chen *et al*., 1986; Shirin & Jamuna, 2010). Furthermore, Stoilova *et al*. (2007) found that ginger CO_2 extract, exerted an antioxidant activity comparable with that of BHT in inhibiting the lipid peroxidation and better than quercetin with regard to hydroxyl radicals inhibition. The Roselle calices are considered as a health drink in many cultural areas. Several reports have demonstrated a strong antioxidant activity of the calices, which is linked to polyphenolic compounds such as anthocyanins and flavonoids (Duh & Yen, 1997; Tsai *et al*., 2002; Chen *et al*., 2004, Hirunpanich *et al*., 2005; Ochani & D'Mello, 2009, Yang *et al*., 2012). Lemon was reported to have the highest amounts of polyphenolic compounds (75.9 ± 3.87 mg/g db [gallic acid equivalents]) among other citrus fruits cultivated in Taiwan, ranging from 36.9 ± 1.84 to 47.0 ± 0.88 mg/g db (gallic acid equivalents) (Wang, Chuang, & Ku, 2007). Different studies focused on antioxidant potency and phenolic composition of tamarind fruits have reported important contents of total phenolic and a good ability to reduce the ion Fe^{3+} and to scavenge radical-cation $ABTS^{\bullet+}$ free radical (Proteggente *et al*., 2002, Sudjaroen *et al*., 2005; Lamien-Meda *et al*., 2008). Passion fruit juice or extract was also found to display 433.5-435 mg GAE/L GA for total phenolic (Talcott *et al*., 2003, Falguera *et al*., 2011) and to have antioxidant activity (Rice-Evans & Miller, 1996; Salah *et al*. 1995) as well as anticancer properties (De Neira, 2003). Regarding all health benefits related above, juices from plants involved in this study must be considered for their polyphenolic compounds contents and antioxidant capacity for functional beverages purposes against chronic diseases (Cardiovascular diseases, cancer, type 2 diabetes) caused by free radicals (Aviram *et al*., 2000; Aviram *et al*., 2004; Rosenblat *et al*., 2006). These troubles, which are considered as diseases of civilization and belonging to industrialized countries, are in emergency in developing country, particularly in sub-Saharan Africa. In Côte d'Ivoire, chronic diseases have become an increasing public health concern. According to WHO (2011), age-standardized death rate per 100 000 for cardiovascular diseases and diabetes is estimated to 547.6 among men and 524.4 among women. Rates for cancers are 80.4 and 78.7 among men and women respectively. In this way, it is necessary to promote functional foods or beverages with antioxidant activity against free radical in order to prevent or to control these diseases. Nevertheless, no study exists on the polyphenols content and antioxidant capacities of the traditional juices consumed in Côte d'Ivoire.

The objective of this study is to evaluate the antioxidant potency of homemade juices currently sold as street beverages in Côte d'Ivoire.

MATERIALS AND METHODS

Experimental materials: Samples of baobab fruit (*Adansonia Digitata* L.), passion fruit, lemon, tamarind pulp, Roselle (*Hibiscus sabdariffa*) calices and ginger (Zingiber officinale (L) Rose) roots juices were bought from women at different markets or in the street. Ten samples were collected for each juice.

Determination of total polyphenols content (TPC): Polyphenols were quantified by the Folin-Ciocalteu (FC) method according to Singleton and Rossi (1965) using a

spectrophotometer (DR 2400, HACH company, USA-Loveland) at 760 nm. Results were expressed in mg of gallic acid equivalents/mL of juices referring to a gallic acid calibration curve (ranging from 3 to 50 µg/mL).

Antioxidant activity determinations: Three methods were used for antioxidant activities measurements. DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) and FRAP (Ferric-ion reducing antioxidant power) assays were performed according to the methods described by Thaipong *et al*. (2006) with slightly modifications.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay: A DPPH methanolic solution at 0.4 mM was prepared. One hundred (100) µL of juice sample were added to 2500 µL of the DPPH solution. After incubation for 30 min at 30 °C in the dark, absorbance was measured at 517 nm using the DPPH methanolic solution as control. Measurements were performed in triplicate. DPPH values were determined from the Trolox standard curve, linear between 25 and 800 µM. DPPH radical scavenging capacity was expressed as Trolox equivalents (in micromolar). Additional dilution was done if the DPPH value measured was over the linear range of the standard curve.

FRAP (Ferric-ion reducing antioxidant power) assay: One hundred (100) µL of each sample was mixed to 2500 µL of FRAP reagent and incubated in the dark condition for 30 min. The FRAP solution was prepared by mixing 25 mL acetate buffer pH 3.6 (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), 2.5 mL TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10 mM TPTZ solution in 40 mM HCl), and 2.5 mL $FeCl_3.6H_2O$ solution (20 mM) and then warmed at 37 °C before using. The absorbance of the coloured product (ferrous tripyridyltriazine complex) was read at 593 nm. The antioxidant capacity based on the ability of sample to reduce ferric ions was calculated from the linear standard curve (25 and 800 µM Trolox) and expressed as Trolox equivalents (in micromolar).

ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) Assay: The radical cation $ABTS^{\bullet+}$ was generated by mixing ABTS (7.0 mM) and potassium persulfate (2.6 mM) and allowing them to stand overnight at room temperature in the dark. The mixture ratio was 1:1 v/v. Then, 1 mL of $ABTS^{\bullet+}$ solution was diluted with 60 mL methanol to obtain an absorbance value between 1.0 and 1.5 at 734 nm. A fresh solution was prepared for each assay. 100 µL of each of juices sample was incubated with 2500 µL of the $ABTS^{\bullet+}$ solution for 2 h in a dark condition. The ABTS free radical-scavenging activity of each sample was calculated from the linear calibration curve (25 and 800 mM Trolox) and expressed as Trolox equivalents (in micromolar).

Statistical analysis: Differences between samples data were tested by ANOVA followed by Turkey's multiple comparison test, using the SPSS software, version 17. Significant difference was determined at 5% ($p<0.05$). An overall antioxidant potency composite index was determined according to Seeram *et al*. (2008). An index value of 100 was assigned to the best score for each test and an index score was calculated for all other samples within the test as follows:

Antioxidant index score) [(sample score/best score) × 100];

the average of all ten samples for each juice was used for the calculation. The overall mean index value was determined by dividing the sum of the individual index by the number of tests (three assays in total: DPPH, ABTS and FRAP). A simple rank order was reported, and where the values were close to each other, an equal rank was assigned.

RESULTS AND DISCUSSION

Total polyphenols content of juices: Total polyphenols content (TPC) of the tested juices is presented in Fig.1. Values are comprised between 3.7 ± 2.49 mg/mL for lemon and 50.1 ± 15.5 mg/L for baobab. TPC decreases in the following order: baobab > Roselle > tamarind > ginger > passion > lemon. The difference in TPC indicates variable number of phenolic groups in these plant materials beverages (Singleton *et al*., 1999).

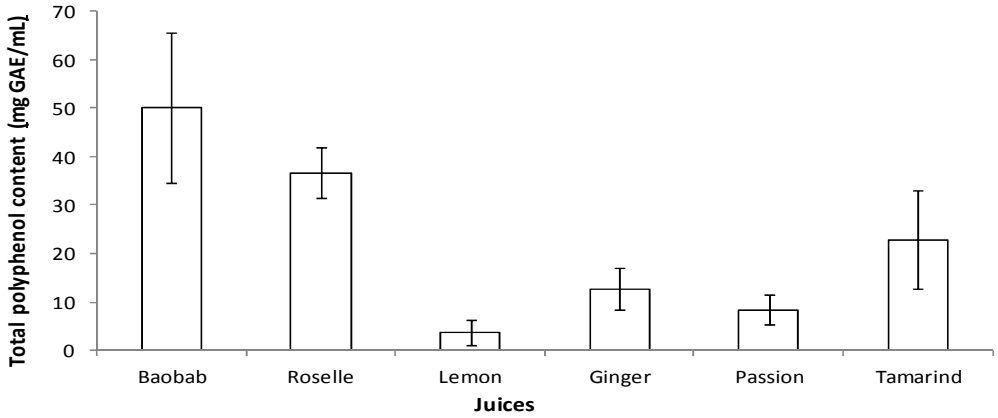

Figure 1: Total polyphenol content (mg GAE/mL) of the different traditional juices

Brady (2011) reported that phenolic compounds present in baobab fruit aqueous extract are flavonoids and probably phenolic acids. Roselle is characterized by its high concentration in anthocyanins (1.5 g/kg^{-1}), delphinidin 3-sambubioside and cyanidin 3-sambubioside are the major (Cissé *et al*., 2009). The common variety of tamarind contains a leucocyanidin pigment (Shankaracharya, 1998) while ginger encloses 6-gingerol and its derivatives. The main phenolic compounds in lemon are flavonoid specially naringin 89.9 ± 2.67 (µg/g db) and luteolin 160 ± 4.45 (µg/g db), and phenolic acid particularly chlorogenic acid (92.6 ± 8.90 µg/g db) and sinapic acid (72.1 ± 2.67 µg/g db) (Wang *et al*., 2007). Flavonoids, mainly C-glycosylflavones, are the major constituents of *P. edulis* pulp with a total flavonoids content equal to158.037 ± 0.602 mg/L (Dhawan *et al*., 2004). Furthermore, except lemon, TPC of the investigated juices was 2 to 29.5 fold higher than amount reported by Seeram *et al*. (2008) for commonly consumed polyphenol-rich beverages in the United States, comprising pomegranate, blueberry, black cherry, açaí and cranberry juices. In addition, the values of TPC were higher than those reported by Falguera *et al*. (2011) for underutilized tropical juices. For example, the phenolic content of passion fruit (433.5 ± 10.2 mg/L) found by the later authors is 20 fold lower than that of the investigated passion juice (8.4 ± 3.07 mg/mL). Lamien-Meda *et al*. (2008) has also reported higher amounts of total phenolics of wild edible fruits from Burkina Faso (West Africa) as compared to the majority of other tropical from Asia.

Antioxidants activities of juices: Antioxidant activities of the investigated juices was tested by DPPH, FRAP and ABTS assays are shown in table 1. All investigated beverages exhibited antioxidant activities with the three methods. Baobab and Roselle juices showed the highest antioxidant potency with the three DPPH, FRAP methods respectively. Values found for these juices are 1132.3±251.6, 1539.2±733.3, 1764.2±234.033 µmol/L and 1137.5±96.03, 1668.7±547.0, 1636.8±117.8 3 µmol/L for DPPH, FRAP and ABTS assays respectively.

Table 1: Antioxidant activities of different traditional juices consumed in Côte d'Ivoire

Juice	DPPH [µmol/L]	FRAP [µmol/L]	ABTS [µmol/L]
Baobab fruit	1132.3±251.6 a	1539.2±733.3 b	1764.2±234.03 a
Passion fruit	493.5±121.1 d	348.5±99.1 e	1352.8±215.1 c
Lemon	231.0±115.9 e	249.2±228.2 f	567.5±266.3 d
Tamarind	836.4±254.5 b	949.6±261.4 c	1713.1±77.9 a
Roselle	1137.5±96.03 a	1668.7±547.0 a	1636.8±117.8 ab
Ginger	773.1±198.2 c	723.9±377.4 d	1557.2±353.7 b

Values with the same letter in the same column are not significantly different

Several studies have shown strong antioxidant activity of Roselle calyx aqueous extract in term of DPPH radical scavenging capacity, ferric iron reduction power and $ABTS^{\bullet+}$ free radical inhibition potency (Duh and Yen, 1997, Tsai *et al*., 2002, Yang *et al*., 2012). This antioxidant property was related to anthocyanins found in the calyx (Tsai *et al*., 2002, Yang *et al*., 2012). Furthermore, Sáyago-Ayerdi *et al*. (2007) found an antioxidant capacity of 335-µmoL trolox equivalents/100 mL Roselle flowers beverage measured by ABTS assay. The result obtained in this study (1636.8±117.8 3 µmol/L) was five times higher than that reported by the later authors. For ABTS assay, in addition to baobab and Roselle, tamarind (1713.1±77.9 µmol/L) presented also high antioxidant activity. Previous studies have shown strong antioxidant activity of aqueous extracts of baobab pulp as DPPH radical scavenging capacity and ferric iron reduction power (Brady, 2011). Nevertheless, the current result shows also $ABTS^{\bullet+}$ free radical inhibition potency. Intermediate values were found for ginger juices with the highest antioxidant activity in the case of ABTS assay. Kishk and El Sheshetawy (2010) reported that the optimum temperature of extraction and reaction time for the maximum radical scavenging activity are 56.12°C and 20.93 min. In Côte d'Ivoire, ginger juice is produced by extraction of grinded roots in tap water without heating. Hence, the antioxidant potency of ginger juice consumed in this country can be improved following the conditions set up by Kishk and El Sheshetawy (2010). Lemon and passion juices displayed the lowest antioxidant capacity with the three assays. Al-juhaimi and Ghafoor (2013) have also found lower DPPH free radical scavenging ability for lemon when compared to orange and mandarin. However, Oboh *et al*. (2012) have reported an intermediate antioxidant activity for packaged lemon juice from Nigeria. In addition, a strong inhibition of DPPH radical was found by Narayanaswamy and Balakrishnan (2011) for aqueous extract (5 g/mL) of passion juice. The difference between these results and those obtained in the present study may due to the concentrations at which the juices or extracts were prepared. This suggests that the antioxidant capacity of traditional lemon and passion juices consumed in Côte d'Ivoire can be improved by adding less water during preparation.

Antioxidant composite index of juices: Table 2 shows that the antioxidant composite index (ACI) of juices was in the following increasing order: Roselle > baobab > tamarind > lemon > ginger > passion. Roselle juice presented the highest index followed by baobab and tamarind. These three juices which showed an antioxidant composite index of 96.1, 89.5 and 74.4 respectively, can be promoted as functional beverages with high antioxidant potency. Nevertheless, Roselle juice is by far the most promising source of antioxidant. Although it is generally known that total polyphenols are highly correlated with antioxidant activity (Seeram *et al*., 2008), some modifications were observed in the trends of total polyphenols content (TPC) and the antioxidant index of the investigated juices. Baobab juice had the most TPC but showed a lower (ACI) than Roselle juice. In addition, lemon presented the lowest TPC but displayed a higher ACI than ginger and passion fruit juices. These results can be explained by difference in the bioactive compounds of the juices. Among the polyphenols of baobab, some may not exhibit antioxidant activities. In addition, apart from the polyphenols, lemon is rich in vitamin C, which also acts as antioxidant (Wang *et al*., 2007).

Table 2: Antioxidant composite index of different traditional juices consumed in Côte d'Ivoire

Juice	DPPH index	FRAP index	ABTS index	Antioxidant composite index
Baobab fruit	99.5	92.2	100	89.5
Passion fruit	43.4	20.9	76.7	32.1
Lemon	20.3	14.9	32.2	44.1
Tamarind	73.5	56.9	97.1	74.4
Roselle	100	100	92.8	96.1
Ginger	68	43.4	88.3	37.1

CONCLUSION

This study shows that among the traditional juices consumed in Côte d'Ivoire, baobab fruit and Roselle calices juices had the highest total polyphenols contents. All the investigated juices exhibited antioxidant activities in term of DPPH radical scavenging potency, ferric ions reducing power, $ABTS^{\bullet+}$ free radical-scavenging ability. Roselle juice exhibited the highest antioxidant capacity with the highest antioxidant composite index (ACI equal

to 96.1), followed by baobab fruit and tamarind pulp juices (ACI equal to 89.5 and 74.4 respectively). These juices are the potential sources of antioxidant with Roselle juice as the most promising source of antioxidant. Therefore, their consumption should be encouraged to prevent or modulate chronic diseases.

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