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Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants

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1 SUMMARY

Powdered fruits or parts of Ivorian plants were obtained after drying and grinding. Water, ethanol, acetone and methanol were tested as extractants of total polyphenols from these powders. The results showed that ethanol was better than acetone, water or methanol for extraction of total phenols from plant byproduct powders. The average total phenol contents of ethanolic, aqueous and methanolic extracts were 9000, 2500, 2000 and 1000 mg Gallic acid equivalent (GAE) / 100 g dry weight in decreasing order (P< 0.05), respectively. Total phenol contents of *Terminalia catappa* (leaves and fruit pulp), *Combretum molle* (leaves), *Arachis hypogea* (skin of the seed), *Annona senegalensis* (leaves) and *Hibiscus sabdariffa* (petals) were 12,000; 11,000; 9,000; 7,000; 6,000; and 4,000 mg GAE/ 100 g dry weight in decreasing order (P< 0.05), respectively and are potential sources of polyphenols (natural antioxidants and nutraceuticals). The other investigated plants had lower phenol contents (~3,000 mg GAE/ 100 g dry weight). Aqueous extracts from the plants were tested for their ability to inhibit α-amylase and α-glucosidase from snail (*Archachatina ventricosa*) digestive tract. Among the extracts tested, five were inhibitors of α-amylase and there was a 7-fold difference between the least and most effective extracts. In order of effectiveness, the potent inhibitors were from *Dioscorea alata* > *Musa paradisiaca* > *Citrus sinensis* > *Terminalia catappa* > *Theobroma cacao*. Comparatively, there was an 8-fold difference between the least and most potent inhibitor of α-glucosidase. The inhibitory effects were in the order of *Solanum macrocarpin* > Solanum distichum > *Solanum melongena* > *Dioscorea cayenensis* > *Arachis hypogea.* These results encourage us to conduct toxicological tests and identify the molecules responsible for these inhibitory effects that have potential application in the management of diabetes mellitus.

2 INTRODUCTION

Polyphenols are molecules from the plant kingdom that represent a wide range of substances with various structures (Antolovich & Robards, 1997).The basic structure is composed of a benzene ring linked to one or more hydroxyl ion, free or involved in another chemical function (e.g. dimethyl ether, ester, sugar). Polyphenols are aromatic compounds formed from the metabolism of shikimic acid and / or that of a polyacetyl. Structurally, they fall into different families including anthocyanins, coumarins, lignins, flavonoids, tannins, quinones, acids and phenols (Wollenweber, 1993, Antolovich & Robards,

1997). This structural diversity results in large variability of the physico-chemical properties influencing the extraction of polyphenols.

The antioxidant activity of fruits and vegetables is generally positively correlated with their content of polyphenols (Hertog *et al.,*1995; Stoner & Mukhtar, 1995; Robards *et al.* 1999; Wang *et al.*, 2000, Wang & Lin, 2000; Benzie, 2003). Polyphenols are a class of compounds that is very broad and complex, of which the flavonoids are the most widely studied.

The interest in polyphenols has grown considerably because of their high capacity to trap free radicals associated with different diseases. Experimental data in vitro suggest that polyphenols have anti-inflammatory, anti-allergic, anti-viral, and anti-carcinogenic activities (Nijveldt *et al.*, 2001; Yan *et al.*, 2002; Boyer & Lui, 2004; Kroon & Williamson, 2005; Jayaprakash *et al.,* 2007). They also have a protective role against cardiovascular diseases (Hertog *et al.*,1995; Silva *et al.*,1997; Prior & Gu, 2005). These biological properties are related to the antioxidant activity of these compounds (Pool-Zobel *et al.*, 1999; Smith *et al.*, 2000).

The extraction of phenolic compounds in plant material is influenced by their chemical nature, the extraction method, the sample size, time and storage conditions as well as the presence of interfering substances (Prior & Cao, 1999). The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process (Perv-Uzulanic *et al.*, 2006).

Côte d'Ivoire, and other countries in the tropical region have a wide variety of exotic fruits and medicinal plants with antioxidant activity, which may have the potential for contributing to sustainable development in this region. This study, to better understand the characteristics of these plants, could lead to the discovery of new sources of antioxidants.

Alpha-Amylases (EC 3.2.1.1) catalyzes the hydrolysis of α-l,4-glucosidic linkages of starch, glycogen, and various oligosaccharides. The inhibition of their activity in the digestive tract of humans is considered to be effective in control of obesity or diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes (Yukiliko & Honda, 1990). Therefore, effective and nontoxic inhibitors of α-amylases have long been sought. On the other hand, polyphenols are known to have strong affinities for peptides or proteins and these characteristics were exemplified and reported as inhibitors of amylase (Ani & Naidu, 2007). However, few studies have yet been done to determine whether the polyphenols of Ivorian plants or their byproducts that are consumed regularly, influence the activities of α -amylase.

The aim of this project was to screen Ivorian plant products to assess the effect of various solvents on the extraction yield of total polyphenols. In addition we investigated how the activities of α -amylase and invertase are affected by the extracted polyphenols

3 MATERIAL AND METHODS

3.1 Plant materials: The powder of different organs of 23 plants was used including the roots, leaves, fruits and stems. The different parts of plants were collected from the market in Abobo (Abidjan, Cote d'Ivoire) or in the vicinity of the University of Cocody in Abidjan taking account of their use in traditional medicine, food and / or other ways (Table 1). The plant part used was washed, cut into thin pieces and dried in an oven at 65 ° C for 72 hrs. The dried material was then pulverized and passed through a sieve mesh of less than 250 µm. The powder obtained was packed in polyethylene bags and stored in a refrigerator at 4 ° C for until further uses.

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Table 1: Names of plants and organs used for extracting polyphenols

Systematic name	Sample number	Part/ byproducts used
Citrus sinensis	1	Frui skin
Terminalia catappa	2	Leaves
Terminalia catappa	3	Fruit pulp
Musa paradisiaca	4	Green skin
Musa paradisiaca	5	Yellow skin
Musa paradisiaca	6	Unripe pulp
Musa paradisiaca	7	Ripe pulp
Borassus aethiopium	8	Fruit pulp
Theobroma cacao	9	Dark chocolate
Dioscorea cayenensis	10	Fermented flour
Dioscorea cayenensis	11	Flour
Dioscorea alata	12	Fermented flour
Dioscorea alata	13	Flour
Glycine max	14	Fermented flour
Albelmochus esculentus	15	Fruit
Solanum distichum	16	Fruit
Solanum macrocarpin	17	Fruit
Solanum melongena	18	Fruit
Hibiscus sabdariffa	19	Petals
Combretum molle	20	Leaves
Annona senegalensis	21	Leaves
Zingiber officinale	22	Roots
Arachis hypogea	23	Roasted seed skin

3.2 Determination of total phenol content: The total phenolics were determined according to the Folin–Ciocalteau method (Rossi, 1965; Waterhouse, 2002; Koffi *et al.*, 2007). About 2 g of plant part or by-product powder was mixed with 8 ml of solvent (water, methanol, acetone or ethanol) and 2 mL of water was added. The tube was capped and shaken at 200 rpm, 60 °C for 30 min in a water bath (gyratory water bath shaker, model G76D, New Brunswick Scientific Co., Edison, NJ). The tubes were removed, vortexed and centrifuged at 2,000 rpm using a Dynac II centrifuge (Becton & Dickinson Company, Franklin Lakes, NJ) for 2 min. The samples were filtered through a 0.45 mm Millipore syringe filter (Whatman, Inc., Clifton, NJ). The total phenolics in the filtrate were determined colorimetrically. A 0.2 N Folin– Ciocalteau reagent (Sigma) was freshly prepared by diluting a 2 N stock solution with water. A volume of 100 mL of filtrate was added to 900 mL of distilled water, and 5 mL of 0.2 N Folin–Ciocalteau reagent was mixed. Saturated sodium carbonate (Sigma Chemical Co.) (4 mL of a 75 g/L solution) was added, followed by mixing with a vortex. The tubes were incubated for 2 h at 25°C and the absorbance was read at 765 nm with a UV-1601 Shimadzu spectrophotometer (Kyoto, Japan). Gallic acid (Sigma) was used to construct a standard curve. The total phenolic content of samples was expressed as milligrams gallic acid equivalents per 100 grams of dry matter (mg GAE/100 g

DM). All measurements were performed in duplicate.

3.3 Extraction of snail digestive juice: The shell of the snail *Archachatina ventricosa* was removed and the digestive juice isolated. By successive finger pressure on the digestive tract, the juice comes out and this was collected in an Erlenmeyer flask. This was the enzymatic extract containing α-amylase and α-glucosidase.

3.4 Determination of reducing sugars: Reducing sugars released during enzymatic hydrolysis of starch and sucrose were assayed by the method of Bernfeld (1955) using the Dinitrosalicylic acid (DNS) assay.

3.5 Determination of α-amylase and α-glucosidase activities: The reaction medium was composed of 50µl of 100 mM acetate buffer, pH 5, 100 µl of substrate starch 1% (w / v) or sucrose 0.5% (w / v) and 100µl of crude enzyme extract (digestive juice of snail).Incubation took place at 37 ° C for 30 minutes. The amount of reducing sugars released was measured by the method of Bernfeld (1955).

3.6 Effects of plants aqueous extracts: The effect of 23 aqueous plant extracts (at the same concentration) on α-amylase and α-glucosidase activities was tested. The concentration of substrate (starch or sucrose) was maintained in the linear portion of the kinetic curve of the enzymes. The amount of reducing sugars released was measured by the method of Bernfeld (1955). The reaction medium contained: 100 µl of 100 mM acetate buffer (pH 5), 100 µl starch, 100 µl of aqueous extract of plants and 50 µl of snail digestive juice. A control was prepared similarly but without plant extract. Then, the reaction mixture was incubated at 37 °C for 10 minutes and the absorbance recorded at 540 nm against a blank. When a plant extract exerts inhibitory effect, activities of enzymes are lower than those obtained in the absence of inhibitor. The inhibition rate was calculated using the formula of Megh *et al.* (2008):

Inhibition (%) = (1 - OD in presence of inhibitor / OD in absence of inhibitor) x100
Where OD is optical density measured at 540 nm

3.7 Statistical analysis: A factorial design was used to compare 4 solvent systems for extraction of polyphenols from 23 plant sources. All measurements and analyses were carried out in duplicates. Analysis of variance (ANOVA) was performed to determine the efficiency of the solvents as well as establish the differences in the content of polyphenols among plant types. The statistical analysis software SAS (Statistical Analysis System) was used. Differences between treatments means were determined by Duncan's procedure at $p< 0.05$.

4 RESULTS

The average total phenol contents of twenty three plant extracts tested for each solvent type is presented in Fig 1. The average total phenols content of ethanolic extracts was significantly different ($p < 0.05$) from aqueous, acetonic or methanolic extracts. The average total phenols contents of ethanolic extracts (9,000 mg GAE : 100 g DM) is at least 4 times higher than that of aqueous or acetonic extracts (2,000 mg GAE g / 100 g DM), and 9 times higher than that of methanolic extracts. Fig 2 shows that among the samples tested, five plant extracts (*Terminalia catappa*, *Combretum molle*, *Annona senegalensis*, *Arachis hypogea* and *Hibiscus sabdariffa*) have significantly higher ($p <0.005$) total phenols content than the other plants. The total phenol contents of plants were in the following decreasing order: *Terminalia catappa* > *Combretum molle* > *Arachis hypogea* > *Annona senegalensis* > *Hibiscus sabdariffa*..

Table 2 contains the results of the inhibition of α-amylase and α-glucosidase from snail digestive tract by aqueous extracts of plants. Among the extracts tested few caused partial inhibition of α-amylase. There was a 7-fold difference between the least and the most

effective extracts. The inhibitory effects of the potent inhibitors were in the order of *Dioscorea alata* > *Musa paradisiaca* > *Citrus sinensis* > *Terminalia catappa* > *Theobroma cacao.* All the other samples had no effect on α-amylase. Comparatively, there was an 8-fold difference between the least and the most potent inhibitor of α-glucosidase. The inhibitory effects were in the order of *Solanum macrocarpin* > *Solanum distichum* > *Solanum melongena* > *Dioscorea cayenensis* > *Arachis hypogea.* All the other samples had no effect on α-glucosidase.

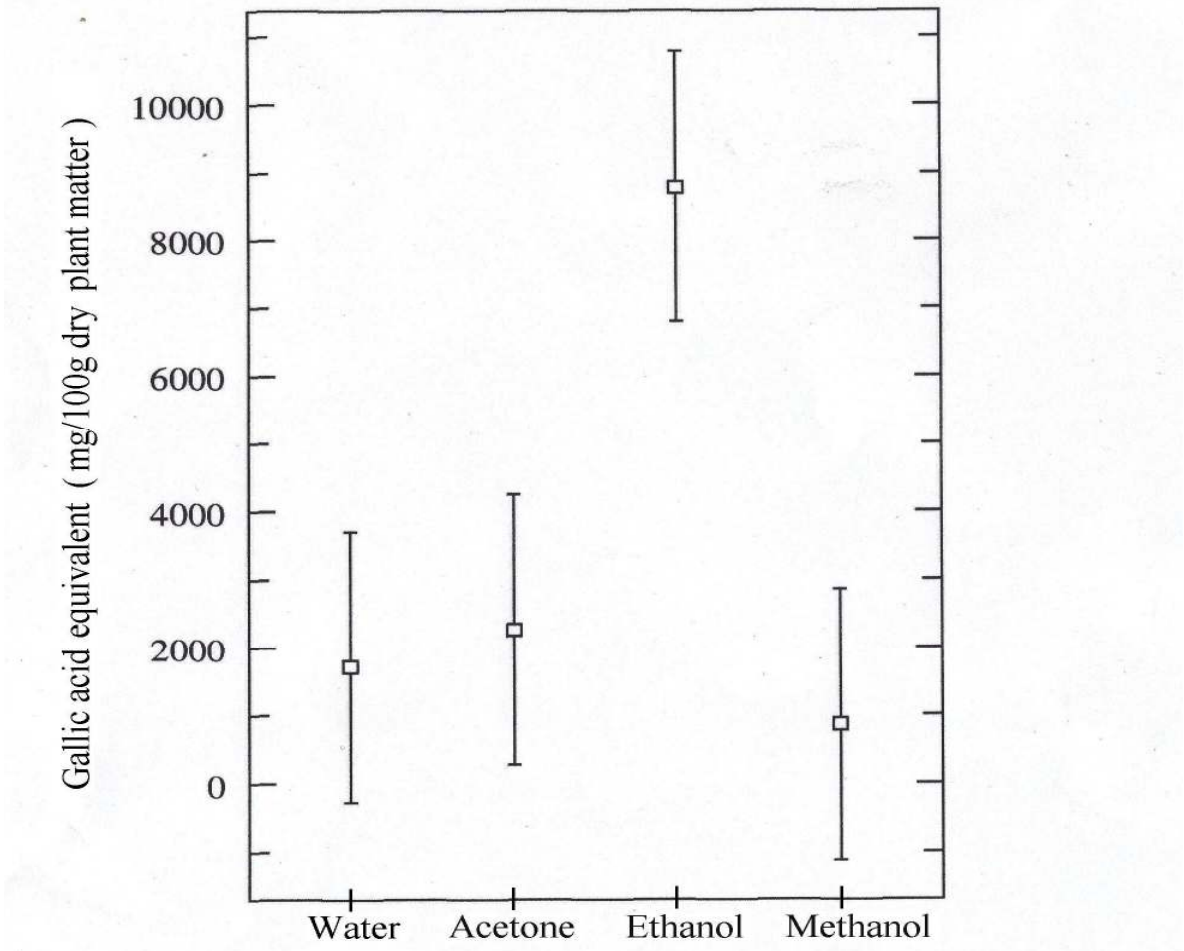

Fig 1. Effect of solvent type on the average total phenol contents of Ivorian plants extracts with 95% confidence interval.

5 DISCUSSION

Methanol, acetone and water are inefficient solvents for extraction of total phenols from powdered plant studied (Fig. 1). The phenolic compounds in the extract are more often associated with other biomolecules (proteins, polysaccharides, terpenes, chlorophyll, lipids, and inorganic compounds) and a solvent must be found that is suitable for extracting them. Research conducted by Jayaprakash *et al.* (2001) confirmed the ineffectiveness of acetone, methanol and water for the extraction of total phenols of grapes seeds (*Vitis vinifera*). However, ethanol / water or acetone / water were better solvents compared to ethanol or acetone (Kalli *et al.* 1995; Yusuf & Toledo, 2006). These authors also showed that the

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methanolic extract was better for catechin, epicatechin and epigallocatechin. The aqueous extraction of plant organs leaves a large amount of residual polyphenols that only an appropriate combination of solvents would extract. It appears from our work that the vast majority of polyphenols are not water soluble. Therefore, to be assured of obtaining fractions rich in polyphenols manufacturers would have to use extraction solvents with a mixture of suitable solvents.

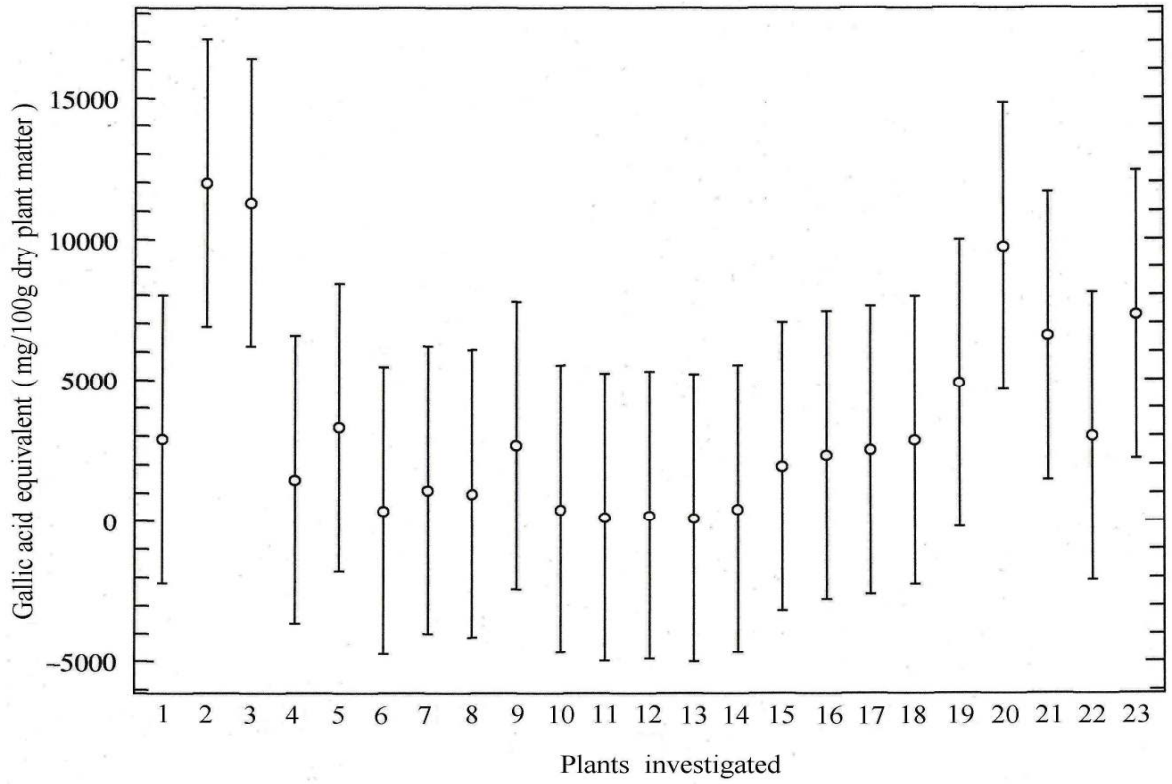

Figure 2: Variation of total phenol contents of Ivorian plants extracts

Figure 2 legend

Plant	Part	Plant	Part
1 : *Citrus sinensis*	Skin	13 : Dioscorea alata	Flour
2 : *Terminalia catappa* :	Leaves	14 : *Glycine max*	fermented flour
3 *Terminalia catappa*	fruit pulp	15 : *Albelmochus esculentus*	fruit
4 : *Musa paradisiaca* L	green skin	16 : *Solanum distichum*	fruit
5 : *Musa paradisiaca* L	yellow skin	17 :*Solanum macrocarpin*	fruit
6 : *Musa paradisiaca* L	unripe pulp	18 :*Solanum melongena*	fruit
7 : *Musa paradisiaca* L	ripe pulp	19 : *Hibiscus sabdariffa*	petals
8 : *Borassus aethiopium*	pulp	20 : *Combretum molle*	leaves
9 :*Theobroma cacao L*	dark chocolate	21 : *Annona senegalensis*	leaves
10: *Dioscorea cayenensis*	fermented flour	22 : *Zingiber officinale*	roots
11: *Dioscorea cayenensis*	flour	23 : *Arachis hypogea*	roasted seed skin
12: *Dioscorea alata*	fermented flour		

The effects of aqueous extracts of plants were investigated in vitro on different enzymes (α-amylase and α-glucosidase) that are involved in the metabolism of carbohydrates. Our results indicate that some soluble compounds of the plant extracts partially inhibit the α-amylase and α-glucosidase, two digestive enzymes that degrade starch and convert it into glucose. These plants therefore are of potential interest in diabetes management because inhibition of the two enzymes mentioned above could slow the digestion of carbohydrates, reducing their absorption and thus limit the rise in blood sugar after meals.

In addition, the inhibitory action of extracts could be enhanced by full recovery of polyphenols using suitable solvents because the affinity of polyphenolic complex is not the same for all types of solvents used (Silva *et al*., 2007). Furthermore, the inhibitory effects of the aqueous extracts were present in two different groups of plants: (*Dioscorea alata*, *Musa paradisiaca*, *Citrus sinensis*, *Terminalia catappa*, *Theobroma cacao L.)* on starch and *(Solanum macrocarpin*, *Solanum distichum, Solanum melongena, Dioscorea cayenensis*, *Harachis hypogea)* on sucrose. This result is due to the fact that several enzyme systems are involved in the hydrolysis of carbohydrates in the snail (*Archachatina ventricosa***)** digestive juice (Soro, 2007).

Table 2: Inhibition test of α-amylase and invertase activities of *Archachatina ventricosa* digestive juice by aqueous extracts of plants investigated.

Plant	**% Inhibition**	
	Sucrose	**Starch**
Citrus sinensis (fruit skin)	0	18
Terminalia catappa (leavese)	0	10
Terminalia catappa (fruit)	0	0
Musa paradisiaca (green skin)	0	0
Musa paradisiaca (yellow skin)	0	31
Musa paradisiaca (unripe pulp)	0	0
Musa paradisiaca (ripe pulp)	0	0
Borassus aethiopium (pulp)	0	0
Theobroma cacao (dark chocolate)	0	8
Dioscorea c. (fermented flour)	5	0
Dioscorea c. (flour)	13	0
Dioscorea alata(fermened flouré)	0	62
Dioscorea alata (flour)	0	0
Glycine max (fermented flour)	0	0
Albelmochus esculentus (fruit)	0	0
Solanum distichum (fruit)	34	0
Solanum macrocarpin (fruit)	43	0
Solanum melongena (fruit)	21	0
Hibiscus sabdariffa (petals)	0	0
Combretum molle (leaves)	0	0
Annona senegalensis (leaves)	0	0
Zingiber officinale (roots)	0	0
Harachis hypogea (roasted seed skin)	5	0

6 CONCLUSION

This study indicated that ethanol is the most efficient solvent for the extraction of polyphenolic components from the Ivorian plants investigated. Among the plants tested, *Terminalia catappa*, *Combretum molle*, *Annona senegalensis*, *Arachis hypogea* and *Hibiscus sabdariffa* have the highest polyphenol contents and are potential sources for this phytochemical. The inhibitory effects of polyphenols on α-amylase and α-glucosidase, two key enzymes involved in the metabolism of carbohydrates may be useful for the management of diabetes mellitus. However, toxicological tests are required before utilization of polyphenol-rich sources in dietary supplements.

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