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Antioxidant potentials of local fruits and foreign wines sold in Ile-Ife, Nigeria

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ABSTRACT

Some locally consumed fruits, sugarcane and wines sold in Ile-Ife, Nigeria, were investigated for their phenol content and antioxidant potential of the methanolic extracts with a view of exploring the health-promoting effect of the fruits and wines. The total phenolic content in each fruit and wines was determined spectrophotometrically according to Folin-Ciocalteu method and calculated as Gallic Acid Equivalent (GAE) while the antioxidant properties of some of the fruits were estimated by the 2, 2-diphenyl-1-picryhydrazyl method (DPPH). The results obtained showed the highest phenolic content in red grape (1.129 ± 0.073 mgGAE/ml) and the lowest in sugar cane (0.160 ± 0.003 mg/GAE/ml). The wines also varied in their phenolic contents with Baron D' Romeo Red wine (0.306 ± 0.014 mgGAE/ml) having the highest phenolic content for the alcoholic wines class and Eva of Spain (0.132 ± 0.002 mgGAE/ml) had the highest phenolic content in the non-alcoholic group. The results also showed that the phenolic content of the alcoholic was greater than that found in the non-alcoholic wines. On the other hand, the antioxidant properties determined in red grape, banana, sweet orange, apple and watermelons showed a change in colour from purple to light yellow instantly in all the fruits except water melon. The study concluded that the fruits are valuable sources of natural phenolic antioxidants which are known to have health promoting properties. Also, that non-alcoholic wines should be improved upon by inclusion of fruits known to contain antioxidant potentials.

Keywords: Antioxidants, fruits, wines, Gallic Acid Equivalent.

INTRODUCTION

Free radicals are unstable molecules that include nitric oxide (NO), molecular oxygen (O_2) and hydrogen atom. Reactive oxygen species (ROS), also called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O^{2-}) and the hydroxyl radical (OH^-), as well as non-free radical such as hydrogen peroxide (H_2O_2). Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides while the endogenous source of ROSs include normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes (Squadrito and Pelor, 1998).

These reactive oxygen species, in an attempt to stabilize, attack other molecules in the body leading to cell damage and triggering the formation of another free radical resulting

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in a chain of reaction. These ROSs have been implicated in several disorders as found in chronic and ageing diseases, rheumatoid arthritis, cataracts, heart diseases, stroke, atherosclerosis, diabetes, cancer and many others (Chen et al., 2004).

The human body has antioxidant defenses against oxidative damages and repair enzymes to remove the ROSs or repair damaged molecules. However, this natural defense mechanism may be insufficient and or inefficient, and hence dietary ingestion of antioxidant compound is important. There have been reports recently that demonstrated that there is an inverse relationship between dietary intake of antioxidant rich diets and the prevalence of human disease (Ali et al., 2001).

Plant phenolic constitutes one of the major groups of compounds acting as a primary oxidant of free radical terminators. High consumption of fruit and vegetables has proven to be associated with lower incidence and mortality rate of various degenerative diseases such as cancer, cardiovascular disease and immune dysfunction. In addition to the vitamins and minerals present in fruit, phytochemicals such as flavonoids and other phenolics may contribute to the protective effects. Many of these phenolic phytochemicals have antioxidant capacity and may protect cells against the oxidative damage caused by free radicals. Phenolic compounds widely distributed in plants, attract significant scientific interest due to their bio-functional health-promoting properties (Rice-Evans et al., 1996).

Fruits are potential sources of natural phenolic antioxidants used as food additives for the prevention of lipid oxidation and thus prolongation of food self-life. Many fruits have been characterized for their phenolic profile and antioxidant activity (Rice-Evans et al., 1996; Vanamala et al., 2006).

The aim of this study is to determine the total phenolic content and antioxidant activities of some commonly consumed edible Nigerian fruits and sugarcane and that of some foreign wines sold in Ile-Ife, Nigeria.

MATERIALS AND METHODS

Materials

Gallic acid (3, 4, 5-trihydroxybenzoic acid), Folin-Ciocalteu's phenolic reagent, 2, 2-diphenyl-1-picrylhyrazyl (DPPH) and Ethanol were purchased from Sigma Chemicals Co. (St. Louis, USA), while Sodium Carbonate, Methanol, Sulphuric acid and Hydrochloric Acid were purchased from BDH Chemical Co. (Poole, England).

Methods

Thirteen (13) different types of commonly consumed Nigerian fruits were bought from the local market in Ile-Ife, Osun State, Nigeria. The fruits were red grape, orange, apple, banana, tangelo, coconut, pineapple, carrot, lemon, garden egg, pawpaw, tangerine, watermelon and sugar cane. Each fruit was thoroughly washed with distilled water. The fruits with outer parts (pericarp) were appropriately peeled off with the aid of a sharp knife. Seventy five grams (75 g) of the fruit was weighed and ground with a mortar and pestle in 25 ml of methanol containing 0.1% HCl. The extracts were filtered over Whatman No: 1 filter paper. The residues were repeatedly extracted in 12.5 ml of methanol containing 0.1% HCl and filtered. All the extracts were combined and the total volume of each extract was measured.

Six different types of foreign wines were also bought from a supermarket in Ile-Ife, Osun State, Nigeria. They are (1) Baron D'Romeo, table Red Wine (10.5% vol.), (Embottlelado por: J. Garcia Caroon, S.A, Ctra. De Murcia, Spain), (2) Barton D' Agoro, table Red Wine (11.5% vol.), (Embottlelado por: J. Garcia Caroon, S.A. Ctra. De Murcia, Spain), (3) Castilo De'liria, 750ml, 12.5% alc/vol, (Embotellado and bottled by: Product et Embotelle per, Vicente gandia, pLA. S.A, Chiva, Valencia, Spain) (4) Donvalls Finest Red Wine, 750 ml, 14% alc./vol, (Vineyard

4il (PTY) Ltd Claremont, 7708, Western Cage, South Africa), (5) Brown and Burk (B&B), 11.5% alc./vol, (Vin Rouge Red Wine, United Manufacturing Europe Limited, R.E CLM 525/CR. 13200E, Product of Spain) and (6) Baron De' Ricot, 13% alc./vol, (Vin De'table, Bottled by: Capel Vinos, S.A, Molino Al fatego, 220 Espinardo, Murcia-Product of Spain). The non-alcoholic wines include (1) Pure Heaven Sprkling Drink, Red Grape Flavour, Produced and bottle in the EU, Sun Mark Limited, England, UB6 8UH, (2) J & W Purple Cocktail, Sparking Soft Drink, Distributed by: Juice and World, S.L Polinjo San Miguel, 31, 132 Villatuerta (Navarra), Spain. (3) Eva of Spain, Sparkling Grape Juice, Produced and bottled in Spain by Envasades Eva, Spain and Flemish Red Sparkling Grape Juice, A product of Belgium, Imported by Mikem, Ganhito-05, B.P 1059, Cotonon, Benin.

Determination of total phenolic content

The total phenolic content of the crude extract of fruits were determined using the Folin Ciocalteu Assay according to the method of Slinkard and Singleton (1977) using Gallic Acid as a standard Phenolic compound.

Preparation of calibration curve

To prepare the calibration curve for the determination of total phenolic content, 0, 1, 2, 3, 4, 5 and 10 ml of the phenol stock solution (5 mg/ml Gallic acid stock solution) were pipette into 100 ml volumetric flasks, and then diluted to volume with double distilled water. These solutions thus have phenolic concentration of 0, 50, 100, 150, 200, 250 and 500 mg/L gallic acid. From each calibration solution, 0.2 ml was pipette into separate test tubes, and to each tubes was added 1.58 ml distilled water, and solution properly mixed. 0.1 ml of Folin-Ciocalteu reagent was added, and the resulting solution was mixed thoroughly. The reaction mixture was left for 8 min and 0.3 ml of the sodium carbonate solution was added. The solutions were left to stand in a thermoregulated water bath at 40 ^{o}C for 30 min. The absorbance values were read at 765 mm against the blank (the '0 ml' solution).

Determination of phenolic content of fruit extracts and wines

A 0.2 ml aliquot of the extracted juice was diluted ten times with distilled water before the addition of 0.1 ml of Folin-Ciocalteu reagent and treated as earlier described. The total phenolic of the samples was interpolated from the linear plot of absorbance against concentration and expressed as Gallic Acid Equivalent of sample extract. Samples were analyzed five times.

For the wine samples, they were all diluted 10 fold i.e. 5 ml of each wine sample was pipetted into a conical flask and 45 ml of distilled water was added. The phenolic content was also estimated as above.

Antioxidant activity test

Qualitative analyses of the antioxidant capacity of five fruits were carried out using DPPH free radical scavenging activity assay (Oke et al., 2002). 0.004% (w/v) of DPPH in methanol was prepared daily and kept in the dark at an ambient temperature. The absorbance of the DPPH solution was measured prior to analysis. The DPPH solution was taken as the control sample. 1, 2, 3, 4 and 5 ml of each fruit extract was pipette into different test tubes followed by serial dilution to make a final volume of 5 ml in each test tube. 5 ml of DPPH solution was then added and the reaction mixture incubated in the dark for 10 min. Absorbance were later read at 517nm. A colour change from purple to light yellow was noted as an indicator of the presence of antioxidant substance in the fruit sample. The experiment was repeated four times.

Statistical analysis

Results are recorded as mean values ± standard deviations. Simple regression analysis was performed to get the line of best fit for the standard solutions used for calibration. The graphic representation was prepared using the graphic software Sigma plot version 7.0

RESULTS

Phenolic content of fruits extracts

The phenolic content in the extracts was expressed as Gallic Acid Equivalent intrapolated from a calibration curve using Gallic Acid as standard as shown in Table 1. The phenolic content of the alcoholic wines are summarized in Table 2 and that of the non-alcoholic drinks in Table 3.

DPPH radical scavenging activity assay

As the DPPH was reduced by the amount of antioxidant present in the sample, the colour change of the solution fade in a proportional correlation to the antioxidant concentration. It was observed that there was rapid change in colour from purple to light yellow upon the addition of DPPH solution to the fruit samples indicating a high concentration of antioxidant in the fruit samples. However, it was observed that watermelon did not give any colour change.

Table 1: Phenolic content of fruit extract.

Fruits	Mean total phenolic content (mgGAE/ml) ± S.D
Red grape	1.129 ± 0.073
Orange	0.963 ± 0.002
Apple	0.665 ± 0.006
Banana	0.561 ± 0.007
Tangelo	0.516 ± 0.017
Coconut	0.508 ± 0.024
Pineapple	0.439 ± 0.005
Carrot	0.439 ± 0.012
Lemon	0.385 ± 0.004
Garden egg	0.344 ± 0.044
Pawpaw	0.298 ± 0.001
Tangerine	0.287 ± 0.011
Water melon	0.287 ± 0.030
Sugar cane	0.160 ± 0.003

The phenolic contents in mgGAE/ml of several fruits and sugarcane found in Ile-Ife, Nigeria. The above results are mean of three independent determinations.

Table 2: Phenolic content of foreign alcohol wines.

Wine	Total phenolic content (mg GAE/ml) ± S.D
Castilo De'Liria	0.204 ± 0.0104
Don valls Red Wine	0.164 ± 0.002
Brown and Burk (B&B)	0.151 ± 0.006
Baron De'Ricot	0.253 ± 0.007
Baron D'Romeo (Red Wine	0.306 ± 0.014
Baron D'Agoro (Red Wine	0.106 ± 0.007

The phenolic contents in mgGAE/ml of several alcoholic wines found in Ile-Ife, Nigeria. The above results are mean of three independent determinations.

Table 3: Total phenolic content of foreign non-alcoholic wine.

Wines	Mean TPC (mgGAE/ml) ± S.D
Pure Heaven Sparkling Drink	0.025 ± 0.003
J & W Purple Cocktail	0.021 ± 0.002
Eva of Spain	0.132 ± 0.002
Flemish Red Sparkling Grape Juice	0.008 ± 0.003

The phenolic contents in mgGAE/ml of foreign non-alcoholic wines found in Ile-Ife, Nigeria. The above results are mean of three independent determinations.

DISCUSSION

In this study, a comparative evaluation of the total phenolic content of the methanolic extract of thirteen (13) different fruits and sugar cane commonly consumed in Nigeria were carried out using the Folin-Ciocalteu method. The antioxidant capacities are related with the content of total phenol. The antioxidant property observed in the fruits extracts can be attributed to the high phenolic content of the extracts. The antioxidant activity of some foods, fruits and vegetables containing phenolic compounds (Karakaya et al., 2001) and of Kei-apple "Dovyalis Caffra" (Du et al., 2001) have been so determined.

The result demonstrated that all the fruit have varying antioxidant potential due to the percentage of their total phenolic content. The antioxidant activities can be due to the presence of secondary metabolites such as volatile oils, carotenoids and vitamins, making some fruits and the foreign wines (averagely) a good source of natural antioxidants for nutritional, medicinal and commercial uses. Phytochemicals have long been recognized to posses many properties including antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic properties (Gulin et al., 2004).

It is observed that the antioxidant properties of foreign alcoholic wine are stronger than that its counterparts, that is non-alcohol wine. The result from this study indicates that some fruits posses strong antioxidant properties which correlate to a reasonable degree with their polyphenolic content. Thus, in order to meet up with the beneficial effects of phenolic compounds, it may be necessary to take at least one or two fruits a day.

On the other hand, it was observed that the total phenolic content of the non-alcoholic wines was small compared to that of the alcoholic wines. The low activity observed in the non-alcohol brands could be due to the fact that the fruit components were not sufficient included. Although, those drinks still serve as beverages, manufacturers are encouraged to include some fruits with antioxidant potentials in them because of their medical importance.

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances in food systems (Cotelle, 2001). DPPH free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid oxidation. The method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. In the DPPH assay, the antioxidants in the fruit samples were able to reduce the stable radical DPPH to the yellow-coloured diphednyl-prcryhydrazine.

Conclusion

The present study determined the levels of phenolic compounds and antioxidant activities among fruits and wines (alcoholic and non-alcoholic) widely present in Ile-Ife Osun State, Nigeria. The major fruits with high antioxidant activities were red grape and orange. These fruits may be considered as good sources of bioactive compounds and could be recommended to people with high incidence of oxidative stress-induced diseases.

Also, alcoholic wines had high antioxidant activities than the non-alcoholic counterpart. An increased but moderate consumption of these alcoholic wines is suggested especially in people with oxidative stress induced diseases such as cancer, cataract, diabetes and atherosclerosis.

Overall, it can be concluded that fruits contains a wide variety of antioxidant phenolics and dietary modification through the balanced consumption of fruits, therefore, is likely to be more important and effective than nutritional supplements for the primary prevention of chronic disease.

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