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(RESEARCH ARTICLE)

Comparative studies on the antioxidant activity of selected alcoholic and non-alcoholic wines commonly consumed in Madonna University, Nigeria

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Abstract

Wines have very essential health benefits due to the presence of compounds that possess antioxidant properties like polyphenols such as anthocyanins, and the body is known to carry out complex metabolic reactions which could release free radicals, hence the need for antioxidants. This study was carried out to understand the increasing intake of wines. A total of four selected wine samples (Don Morris, Baron Romero, Capel, Raim D'Or) were analyzed to evaluate their antioxidative capability. A low IC50 value means high scavenging activity by the wine sample. From the results obtained, it was observed that Don Morris had the highest inhibition activity in relation to nitric oxide radical, when compared against the control Sodium nitroprusside (SNP) only. The IC50 for superoxide anion inhibition was significantly low in Don Morris wine (1.63 ± 0.09%) when compared with Raim D'Or wine (18.31 ± 4.16%). Furthermore, in the DPPH radical scavenging assay Don Morris wine had a low IC50 value of (0.63±0.02%) compared to Raim D'Or with (1.31 ± 0.16%), indicating a more efficient DPPH radical inhibiting activity. Conclusively, Don Morris showed high inhibitory effect of DPPH, Nitric oxide and Superoxide anion free radicals, when compared with the other wines used for the research. Therefore, to avoid or manage oxidative stress and its related diseases, it is recommended that Don Morris wine be taken as it has shown its potential in the inhibition of free radicals hence higher antioxidant activity when compared with Baron Romero, Capel and Raim D'Or wines.

Keywords: Antioxidant wines; Free radicals; Scavenging activity; Antioxidative capabilities; Polyphenols

1. Introduction

The body is known to carry out complex metabolic reactions that take place every second and minute to facilitate the various multiple needs of the body. Most of these reactions involve the need for conversion of a certain dietary or body synthesized substance to a form in which the body can effectively use. Such reactions include for example, the breakdown of any carbohydrate rich meal into the common form of glucose (a simple sugar) which goes through a series of reactions to produce energy in form of adenosine triphosphate (ATP) which is used to drive chemical reactions like the synthesis of nucleic acid. Most reactions have different mechanisms or mode of action by which they carry out their duties. Oxidation is a chemical process that can produce free radicals leading to chain reactions that may damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves [1].

The French Paradox is based on epidemiological studies that report a comparatively lower incidence of coronary heart disease (CHD) in France despite high levels of saturated fat in the traditional French diet. A moderate daily consumption of red wine has been proposed to contribute to this effect [2]. The mechanisms responsible for the healthful effects of wine are extremely complex. Both the alcohol and the polyphenol components have been extensively studied and there

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is controversy over which component is more important [3, 4]. The strong antioxidant properties of wine are mainly due to the presence of a large amount of polyphenols and anthocyanins as major families of the compounds having antioxidative properties in red wine [5, 6]. This study hence was designed to study the antioxidant ability between non-alcoholic and alcoholic wines and reach a logical conclusion on which one has more oxidant scavenging ability.

2. Materials and methods

2.1. Chemicals

All the chemicals used in this study were of analytical grade. The solvent ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, Methionine, Riboflavin, Ethylene diamine tetra acetic acid (EDTA), Nitro blue tetrazolium (NBT), Phosphate buffer, Sodium chloride (NaCl), Disodium hydrogen phosphate, Sodium nitro prusside (SNP), Sulfanilamide, N-naphthyl ethylene diamine, Phosphoric acid, Sodium nitride, Potassium buffer, Potassium dihydrogen phosphate (KH¬2PO4) were all purchased from Sigma Chemical Co.

2.2. Equipment

All the equipment used in this research experiment was available at the Department of Biochemistry Madonna University, Nigeria. The equipment used during this research was an Agilent 8453E UV-visible spectrophotometer for reading the absorbance of each sample mixture and an oven for drying the test tubes before each assay.

2.3. Sample used

Radical scavenging assays were used to test each wine sample (Capel, Raim D'Or, Don-morris and Baron Romero). This was to ascertain the level at which each of the wine samples are able to exhibit their antioxidant capabilities by inhibiting the free radicals.

The wines used for the experiment are found commonly in Madonna University; a brief selection of about four wines often taken were selected. The wines are; Capel (Non-alcoholic), Raim D'Or (Non-alcoholic), Don-morris (Alcoholic), Baron Romero (Alcoholic). Serial dilution was done for each wine consisting of 10 test tubes including a blank and control for various radical scavenging assays.

Procedure for serial dilution used in each test;

i) 80% ethanol was prepared from combining 20 ml of water into 80 ml of ethanol.
ii) Test tubes were labeled from 1 – 10 and then control and blank.
iii) 2.0 ml of ethanol was pipetted into each test tube except the test tube labeled 1.
iv) 4.0 ml of the wine was pipetted into test tube labeled 1 which served as 100% of the wine sample, pure and not mixed.
v) 2.0 ml of the wine sample in test tube 1 was extracted and imputed into test tube 2 which is 50% of the wine.
vi) 2.0 ml of the wine sample in test tube 2 is extracted and imputed into test tube 3.
vii) 2.0 ml of the wine sample from test tube 3 is extracted and imputed in test tube 4.
viii) Extraction of wine sample from one test tube to the next continues until test tube 10 where 2.0 ml is extracted and discarded.
ix) For the test tube labeled control and blank had 2.0 ml of ethanol each.
x) The test tube labeled control had the 2.0 ml of ethanol and 1.0 ml of assay reagents (DPPH, Superoxide anion, Nitric oxide).
xi) The test tube labeled blank had 2.0ml of ethanol and 1.0 ml of wine.

2.4. Antioxidant assays

2.4.1. DPPH radical - scavenging assay

DPPH radical scavenging activity was detected for antioxidant activity by in vitro assay. The principle of reaction is quantitative. DPPH is lilac purple in colour; hence a substance with antioxidant activity changes from purple to yellow. Chemically, this change occurs when the sample substance can donate one proton (H^+) to the free radical then it is said to have antioxidant scavenging property. 2.0 ml solution of the wine sample at different concentrations (25%, 35%, 40%, 45% & 50%) diluted in ethanol was mixed with 1.0 ml of 0.3 mM DPPH in ethanol. The mixture was covered with

aluminum foil paper, shaken vigorously and allowed to stand at room temperature in the dark for 30 minutes. Blank solutions were prepared with each wine sample solution of 2.0 ml and 1.0 ml of ethanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of ethanol. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with an Agilent 8453E UV-visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\%\ Inhibition\ = 100\ \% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested wine sample. The IC_{50} value represented the concentration of the wine sample that caused 50 % inhibition of DPPH radical and was calculated by linear regression of plots, where the abscissa represented the concentration of tested sample and the ordinate the average percent of inhibitory activity from four replicates.

2.4.2. Superoxide anion inhibition

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) [7]. The principle of reaction is similar to the DPPH radical scavenging assay as it is also quantitative. Superoxide anion is blue in colour therefore when the solution is exposed to the fluorescent light if the solution has antioxidant abilities; the blue colour formation is inhibited. Briefly, each 3.0 ml reaction mixture contained 0.05 M (1 ml) phosphate buffered saline (PBS) (pH 7.8), 13 mM (390 µl) methionine, 2 µM (60 µl) riboflavin, 100 µM (300 µl) EDTA, 75 µM (200 µl) NBT and 1.0 ml of wine sample solutions (10–250 µg/ml). The tubes were kept in front of a fluorescent light (725 lumens, 34 watts) and absorbance was read at 518 nm after 20 minutes. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes containing reaction mixtures were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was estimated by comparing the absorbance of the control and those of the reaction mixture containing test sample as per the equation:

$$\%\ Inhibition\ = 100\ \% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

2.4.3. Nitric oxide radical scavenging assay

Nitric oxide (NO) generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al*. (2005) [8]. Briefly, the reaction mixture contained 1.0 ml of SNP, 1.0 ml of wine sample and vitamin E (positive control), 6.0 ml of phosphate buffered saline (pH 7.3) in the first sample group, the second sample group contained 1.0 ml of SNP, 2.0 ml of wine sample and vitamin E (positive control), 5.0 ml of phosphate buffered saline. In the control; 1.0 ml of SNP, 7.0 ml of phosphate buffered saline. The mixture was incubated at 25°C for 30 minutes in front of a visible polychromatic light source (25 Watts tungsten lamp). The Nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion (NO_2^-) which was assayed at 30 minutes intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% N-naphthylethylenediaminedihydrochloride).

The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-naphthylethylenediaminedihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the wine sample was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out twice.

3. Results

3.1. DPPH radicals inhibitory activity

The sampled wines showed significant concentration-dependent DPPH radical scavenging capacity as shown in Table 1. Don Morris wine was the most efficient DPPH radical inhibitor, inhibiting 92.95 ± 2.74 % of DPPH at a concentration of 6.25 % v/v compared to Capel wine which inhibited 90.51 ± 0.42 %, Raim D'Or which inhibited 72.51 ± 15.42 % and Baron inhibited 86.95 ± 6.74 % at same concentration. The IC_{50} values for DPPH radical inhibition corroborated the

radical inhibitory potential of the wines in the order Don Morris < Capel < Baron Romero <Raim D'Or. Raim D'Or therefore showed the least radical scavenging capabilities since lower IC50 indicates good activity.

Table 1 DPPH radical scavenging activity of wine samples

Concentration (% v/v)	Capel	Raim D'Or	Don Morris	Baron Romero
6.25	90.51 ± 0.42	72.51 ± 15.42	92.95 ± 2.74	86.95 ± 6.74
3.13	79.43 ± 2.54	60.43 ± 24.54	75.10 ± 2.35	78.10 ± 2.35
1.56	69.16 ± 1.51	53.16 ± 37.51	66.91 ± 3.95	66.91 ± 1.95
0.78	61.73 ± 6.58	41.73 ± 46.58	57.07 ± 5.96	54.07 ± 3.96
0.39	48.19 ± 2.06	14.19 ± 52.06	40.70 ± 5.53	44.70 ± 5.53
0.19	14.09 ± 2.95	9.09 ± 32.95	18.18 ± 3.77	28.18 ± 2.77
IC_{50}	0.71 ± 0.07	1.31 ± 0.16	0.63 ± 0.02	0.65 ± 0.06

Data represented as mean ± SEM (n = 2)

3.2. Inhibitory effect of wine on superoxide ($O_2^{\cdot-}$) anion radical

The wine samples inhibited the formation of superoxide anion radicals in a concentration-related manner. As shown in Table 2, the Don Morris wine was the most potent superoxide anion inhibitor, showing the maximal $O_2^{\cdot-}$ anion inhibitory activity of 74.95 ± 8.74 % at the concentration of 12.50 % v/v, compared to Capel wine (66.75 ± 1.82 %); Raim D'Or wine (44.51 ± 5.40 %) and Baron Romero (47.91 ± 2.14 %) at the same concentration. The IC_{50} for $O_2^{\cdot-}$ anion inhibition was lowest for Don Morris wine (1.63 ± 0.09 %v/v) and highest for Raim D'Or wine (18.31 ± 4.16 % v/v)

Table 2 Superoxide anion radical ($o_2^{\cdot-}$) inhibition by wines

Concentration (% v/v)	Capel	Raim D'Or	Don Morris	Baron Romero
12.5	66.75 ± 1.82	44.51 ± 5.40	74.95 ± 8.74	47.91 ± 2.14
6.25	46.51 ± 4.41	34.51 ± 1.42	65.95 ± 2.70	38.09 ± 1.77
3.13	37.43 ± 3.50	28.43 ± 2.59	57.10 ± 4.35	32.10 ± 2.35
1.56	24.16 ± 1.58	18.16 ± 3.51	44.91 ± 5.95	28.91 ± 1.95
0.78	19.73 ± 2.54	10.73 ± 4.58	33.07 ± 3.96	21.07 ± 3.16
0.39	9.19 ± 0.16	5.19 ± 0.86	21.70 ± 1.53	14.70 ± 1.53
IC_{50}	9.99 ± 1.77	18.31 ± 4.16	1.63 ± 0.09	15.60 ± 2.06

Data represented as mean ± SD (n = 2)

3.3. Inhibitory effect of wine on nitric oxide ($NO^{\cdot}$) radical generation

This study showed that the wine samples in SNP solution decreased levels of nitrite, a stable oxidation product of $NO^{\cdot}$ liberated from SNP (Fig. 1). The wines exhibited best $NO^{\cdot}$ radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The $NO^{\cdot}$ radical inhibitory activity among the wines was in the order Don Morris > Baron Romero >Capel>Raim D'OR compared to the control SNP only.

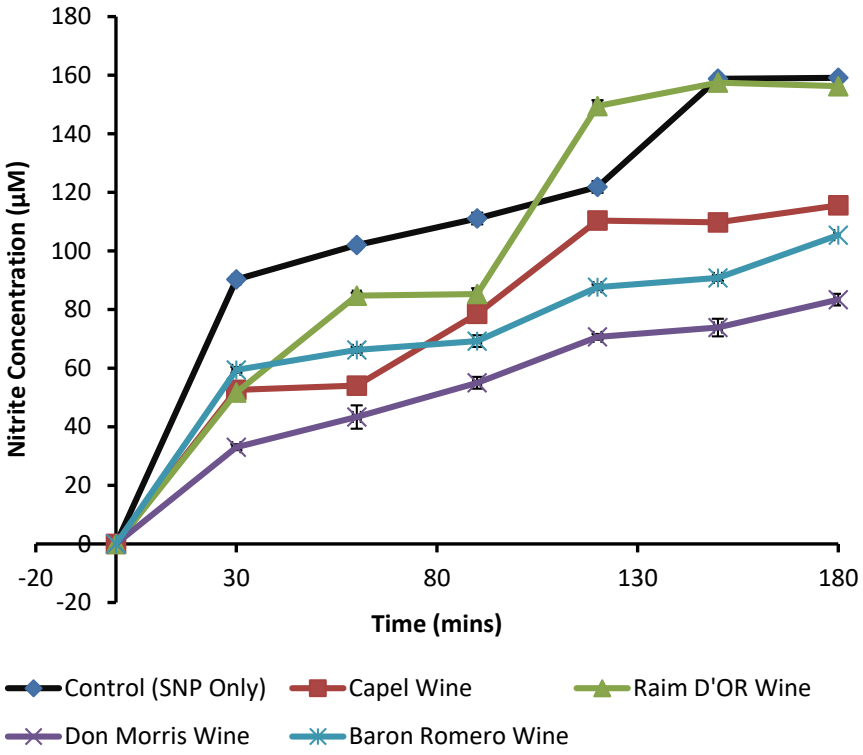

Figure 1 Effect of wines on the accumulation of nitrite upon decomposition of SNP (5 mM) at 25 °C. Each plot represents the mean ± SEM (n = 2).

4. Discussion

Antioxidants are molecules that are generated by the body and also ingested via dietary sources majorly fruits and vegetables such as green leafy vegetables, nuts which have high quality fiber; cashew nuts, walnuts, berries, apples and more [9].

Wines are liquid beverages made from different variety of grapes such as pinot noir, cabernet sauvignon, and merlot: for red wines. The strong antioxidant properties of wine are mainly due to the presence of a large amount of polyphenols and anthocyanins as major families of the compounds having antioxidative properties in red wine [5, 6]. These polyphenols contain antioxidants that scavenge free radicals in the body.

The focus is on comparing the wines to evaluate or ascertain their antioxidant capabilities. DPPH is chemically known as 2, 2-diphenyl-1-picryhydraxyl which is composed of stable free-radical molecules [10]. DPPH is most notably applied in common antioxidant assay as a scavenger for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction [11]. The IC_{50} values for DPPH radical inhibition showed the order of radical inhibition to be Don Morris (0.63 ± 0.02) < Capel (0.71 ± 0.07) < Baron Romero (0.65 ± 0.06) <Raim D'Or (1.31 ± 0.16) which indicated no significant difference between Don morris, Baron Romero and Capel but a significant difference when compared to Raim D'Or. According to the research carried out by Busuricu *et al.,* (2008) [12] based on the Dimethyl-4-phenylenediamine (DMPD) method used to measure antioxidant power, it is observed that the red wines have a higher antioxidative activity than the white wines. The difference of antioxidative activity is explained on the basis of the different antioxidative compounds they contain. The IC_{50} calculated indicates that a lower IC_{50} shows a high scavenging activity by the wine samples. Superoxide anion radical is particularly important as the product of the one-electron reduction of di-oxygen O_2, which occurs widely in nature [13]. One approach that has been used in quantitative assays converts superoxide to hydrogen peroxide, which is relatively stable. Hydrogen peroxide is then assayed by a fluorimetric method [14]. The IC_{50} values for superoxide anion inhibition showed the order of radical inhibition to be Don Morris (1.63 ± 0.09) < Capel (9.99 ± 1.77) < Baron Romero (15.60 ± 2.06) <Raim D'Or (18.31 ± 4.16) which indicated no significant difference between Baron Romero and Raim D'Or, but a significant difference when compared to Don Morris. Nitric oxide is a free radical produced when sodium nitroprusside

(SNP) is used in an assay medium to detect or determine the antioxidant activity levels by the ability of the wine samples to depress the nitrite activity in the medium. This protects against oxidative damage. According to the graph in Figure 1, the absorbance of the assay medium was compared to the standard control which contained SNP only in relation to time. From the results observed, Raim D'Or being the closest to the control exhibited a low inhibition activity while Don Morris had the highest inhibition activity in relation to the nitric oxide radical. The range of inhibition activity observed was Don Morris >Baron Romero>Capel>Raim D'Or.

In comparing the alcoholic and non-alcoholic wines and their capabilities, it is observed that alcoholic wines have better antioxidant capability than non-alcoholic wines.

5. Conclusion

The antioxidant assays carried out clearly showed that wines do contain antioxidant or exhibit radical scavenging activities however, the levels at which antioxidants are present in a wine determines the level at which the wine inhibits or scavenge for free radicals. From the results obtained, it shows that Don Morris has shown its potential in scavenging DPPH, Nitric oxide and Nitrite free radicals, when compared with the wines and could be useful for prevention or management and treatment of oxidative damage and other free radical related diseases such as cancer, heart disease etc.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest

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