Arabian Journal of Chemistry (2016) **9**, 1–8

King Saud University

Arabian Journal of Chemistry

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REVIEW

Comparative study of the anti-oxidant activity of the total polyphenols extracted from *Hibiscus Sabdariffa* L., *Glycine max* L. *Merr*., yellow tea and red wine through reaction with DPPH free radicals

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Received 27 February 2013; accepted 14 November 2014
Available online 18 December 2014

KEYWORDS

Glycine max;
Sabdariffa Hibiscus;
Yellow tea;
Red wine;
Polyphenols;
Antioxidant

Abstract The present study is part of the evaluation of extracts *of Glycine max* L. *Merr* and *Hibiscus* L. *Sabdariffa* as antioxidants. A comparative study was performed with extracts of yellow tea and commercial red wine, two foods known for their antioxidant activity. The method applied is free radical scavenging using the 1,1-diphenyl-2-picrylhydrazyl (DPPH°). The antioxidant properties were identified and measured by the determining the anti-radical activity reducing index, expressed in percentage % RSA (Radical Scavenger Activity), and by the determination of the colouring intensity (IC50). All results are compared to those of ascorbic acid as reference antioxidant. The results indicate the following order for the antioxidant power of the extracts tested. % RSA (tea) > % RSA (*Glycine max*) % > RSA (red wine) % > RSA (Sabdariffa Hibiscus), and colouring intensities (IC50) ranging from 4.62 μM (ascorbic acid) to 1.10 μM (*Hibiscus sabdariffa*) correlated with their chemical structure and the content of phenolic compounds.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.11.048

Contents

1. Introduction

The terms antioxidant and free radical are popular expressions used by nutritionists and other healthcare professionals. In recent years, much information has appeared about the role of oxidative stress in the development of a number of serious illnesses, such as certain cancers, cardiovascular diseases and age-related degenerative diseases, and about the possible therapeutic value of antioxidants against these illnesses.

The importance of vegetables, fruits, legumes and berries as part of a healthy diet is generally accepted. One possible reason why these foods promote good health could be the presence of a range of antioxidants in edible plants, for example vitamins C and D, carotins, selenium, folates and phenolics compounds, including flavonoids.

To date, over 3000 flavonoids have been identified. These can mainly be found in the pigments in flowers or in leaves (Marfak, 2003). Flavonoids are primarily known for their anti-oxidative (Bruneton, 1999), vasculoprotectrice (Vitor et al., 2004; Ghedira, 2005), anti-inflammatory (Chen et al., 2008) and antidiabetic (Marfak, 2003) properties. Antioxidants are molecules which can interact with free radicals and break the chain reaction before vital molecules are damaged (Evans, 2007).

Free radicals are chemical species with one or two unpaired electrons in their outermost layer, which can be created in a multiple ways. They can be exogenic (e.g. ultraviolet radiation, pollution, infections, tobacco) or endogenic. A lack of antioxidant or an overproduction in free radicals can lead to an imbalance between the oxidant and antioxidant system. One of the most significant factors in the production of free radicals is oxidative stress (Guerci et al., 2001; Punitha et al., 2005; Zhao et al., 2005; Long et al., 2004). Oxidative stress is involved in a several illnesses, including diabetes (Pincemail et al. 1999; Huang et al., 2004), atherosclerosis, Alzheimer's disease, Parkinson's disease, glaucoma and age-related macular degeneration (Drobek-Slowik and Karczewicz, 2007; Bonne and Muller, 2000). The provision of antioxidants through diet is a simple means to reduce the development of illnesses brought on by oxidative stress (Zafra-Stone et al., 2007; Bagchi et al., 2000).

Polyphenols are well know that antioxidants (Fraga, 2007). They transfer an electron to the free radicals, which thus become stable as their electrons are paired. This prevents damage to cells and tissue caused by oxidant stress. Consequently, a diet which is rich in polyphenols could modulate certain secondary physiological effects of oxidant stress and prevent obesity (Prior and Wu, 2006) or optimise the treatment of diabetes (Bagchi et al., 1997; kim et al., 2002). *Glycine max* (soya) and *Hibiscus sabdariffa* (roselle) are two edible plants which are rich in anthocyanins.

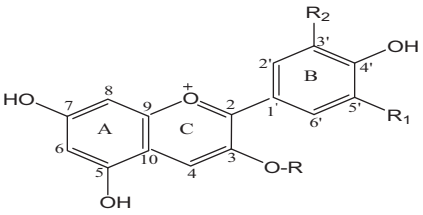
The present study is intended to contribute to the valorisation of African plants through determining their antioxidant activity. The total polyphenol extracts present in these two plants were tested with 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH°), which is a free radical that has been used in several scientific studies (Athamena et al., 2010; Ba et al., 2010; Cristina et al., 2009; N'gaman Kohué et al., 2009; Sharma and Bhat, 2009). The aim of the present study was to compare the anti-radical effectiveness of these two plants with the recognised antioxidants red wine and tea (Bourzeix, 1993; Katiyar, 1999; Changotade et al., 2007; Yusuf, 2007; Yao et al., 2008; Camouse et al., 2009) by determining the relative reduction in DPPH° radicals and the necessary quantity of antioxidant required to reduce DPPH° by 50%. The results are compared with those of ascorbic acid as a reference antioxidant.

2. Materials and methods

2.1. Materials

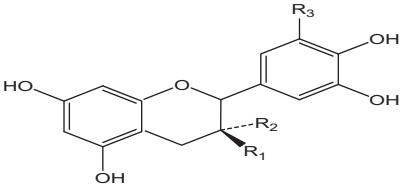
2.1.1. The plants

Hibiscus sabdariffa L. is a perennial herbaceous plant found to the tropical and subtropical zones of both hemispheres. The species is grown for its fibres and calyces, of which there are three types: green, red and dark red. The red calyces are the most commonly used type. They contain a high concentration of anthocyanins which can reach 1.5 g/kg (Mazza and Miniati, 2000). Delphinidin-3-sambubioside and cyanidin-3-sambubioside are the major anthocyanins with 71% and 29% total anthocyanins respectively. Due to their high content in acids, vitamin C and particularly anthocyanins, the red calyces are the most used part of the plant (Kerharo and Adam, 1974; Morton, 1987; Babalola et al., 2001; Wong et al., 2002; D'Heureux–Calix and Badrie, 2004). They are eaten as a vegetable and are used in tonic drinks and traditional medicine.



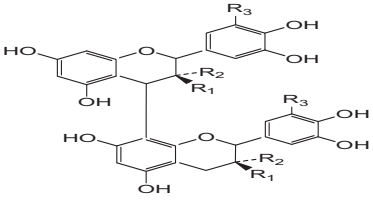
R1	R2	R	Anthocyanidins
OH	H	H	Cyanidol
OH	OH	H	Delphininol
OCH_3	OH	H	Petunidol
OCH_3	H	H	Peonidol
OCH_3	OCH_3	H	Malvidol

Figure 1 Structures of anthocyanidins in grape, red wine, *Glycine max* and *Hibiscus sabdariffa* (Cheynier et al., 1998).



R1	R2	R3	Structure name
OH	H	H	(+) -catechin
H	OH	H	(-) - epicatechin
OH	H	OH	(+)-gallocatechin
H	OH	OH	(-) -epigallocatechin

Figure 2 Structures of the monomers of red wine tanins and tea polyphenols (Cheynier et al., 1998).



R1	R2	R3	Structure name
H	OH	H	Procyanidols
H	OH	OH	Prodelphinidols

Figure 3 Structures of the grape tanins (Cheynier et al., 1998).

Glycine max L. Merr. or soya, belongs to the fabaceae family – more commonly known as legumes. It grows to about 80 cm in height. After blossoming of its red, mauve or white flowers, it develops pods which contain between 2 and 5 beans, which are used as a foodstuff. Studies have shown that soya is rich in phenolics compounds, particularly anthocyanins (derivates of cyanidin, delphinidin and petunidin) (Andzi Barhé, 1998). Consequently there is much interest in the consumption of this plant and its effects on health.

2.1.2. The beverages

For the study a retail red wine was used with 12% alcohol in a 750 mL volume (RE. N° 4483–MU/2). The tea was of a common household brand sold in 2 g bags. It should be noted that the colloidal structure of red wines is linked to the presence of the main groups of components – glucidic and phenolic components.

In grapes, phenolic components are found in high concentrations in the skins and seeds (Ribéreau-Gayon, 1972; Bourzeix et al., 1986). Polyphenols are notably the cause of the colour of red wines, and they play a role in the organoleptic characteristics of the wine, due to their structure as well as their concentration. The phenolic compounds are mainly: flavonoids, which include flavonols, anthocyanins and flavan-3-ols, including catechins and epicatechins and their glycosyled derivatives, condensed tannins (Lacopini et al., 2008). Several studies have shown that yellow tea, like green tea, is composed of approximately 40% catechins, including epicatechin, epigallocatechin, epicatechin-3-gallate (Katiyar, 1999; Changotade et al., 2007).

Of these, epigallocatechin-3-gallate plays a particularly important role in the prevention of photoaging and the prevention of cancers (Kim, 2001). The structures of the polyphenols in *Glycine max*, *Hibiscus sabdariffa*, tea and red wine are shown in Figs. 1–3 below.

2.2. Methods

2.2.1. Extraction of the total polyphenols

250 g of dried *Glycine max* seeds and 5 g of *Hibiscus sabdariffa* were soaked in 250 mL of a solution of ethanol and 1% trifluoroacetic (EtOH–TFA 1%) at 5 °C for 72 h. The extraction occurs in acidified solvents in order to stabilise the anthocyanins, which are very instable species in neutral and alkaline environments. The method is described in the works of Ribereau Gayon, 1968; Oleszek et al., 1994; Yen and Chen, 1995 and Lin et al., 1996.

The extract is filtered, and then concentrated under reduced pressure at 40 °C until it is almost dry. It is then mixed with 15 ml of water and shaken well to produce a concentrated

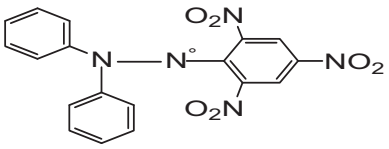


Figure 4 Chemical structure of the 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH°).

filtrate. This is then purified by column chromatography using an Amberlite XAD-7.

The total polyphenol extracts are obtained after degreasing the column with 250 mL of 0.05% solution of trifluoroacetic (TFA 0.5%) and elution with 70% ethanol (EtOH 70%). The éluât is concentrated under vacuum at a temperature below 40 °C and then lyophilised.

250 mL of red wine was taken as a sample. After evaporation of the alcohol at a temperature below 40 °C, the extract was diluted in acidified water and then filtered. The filtrate is treated with the same steps as the extracts of *Glycine max* and *Hibiscus sabdariffa* to produce the lyophilisate. For the tea, the same procedure was followed, using 2 g of tea. However, the extraction was undertaken with 250 mL of heated water below 40 °C. After cooling, the infusion is filtered, and then fixed on Amberlite as described above to obtain the total extracts and the lyophilisate.

2.2.2. Measurement of the antioxidant activity

Two approaches were used to determine the antioxidant activity. In the first, the activity was determined by the indices of reduction of the Radical Scavenger Activity in percent (% RSA), of the absorbance of the reactional mixture which contains the free radical and the sample of antioxidant is linked with the absorbance of the mixture without antioxidant (control solution) in the time t, where

$$\%\mathrm{RSA} = [(A_T - A_E)/A_T] \times 100$$

A_T: absorbance of the control (DPPH° only). A_E: absorbance of the test subject (extract + DPPH°)

The relative index % RSA only indicated the capacity of the sample, at a given concentration, to reduce the radicals, and in many cases the increase in concentration of the antioxidant leads to an increase in the relative indices (Sanchez-Moreno et al., 1998).

To eliminate the influence of the concentration, the second approach is to estimate the reactivity by determining the colouring intensity IC50 of each antioxidant. The IC50 is the concentration (in mol/L) of DPPH corresponding to the optical change in optical density caused by a change of 50 ppm of the antioxidant. The antioxidant capacity of compounds is higher, when the colouring intensity (IC_{50}), is higher. IC_{50} is determined by the ratio

$$IC_{50} = (50|\mathrm{pente}|C_{DPPH})/DO_{DPPH}$$

where C_{DPPH}: concentration of DPPH in mol/L. DO_{DPPH}: Absorbance of the control tube (DPPH° only).

The incline is given by the change of the absorbance in function of the concentration. In both approaches the activity is measured using the methods described in (Chang et al., 1993; Wahllandir et al., 1979; Awika et al., 2003) and numerous other authors (Huh et al., 2004; Makris et al.,2007; Bakkalbas et al., 2005). The radical DPPH° is dissolved in methanol with a concentration of 8.57×10^{-4} mol/L and maintained out of the light at −20 °C before use. Eight test tubes were prepared, of which seven contains increasing concentrations of the test extract. The solutions were prepared by dissolving 112 mg of lyophilisate in 100 mL of methanol. 3 mL of DPPH° was added to each tube, and the absorbance was measured after 10 min with a CIBA CORNING 2800 spectrometer at 517 nm. The total volume in each tube is 3.5 mL.

1,1-diphényl-2-picrylhydrazyl free radical used to study relationship between the structure and antioxidant activity of the phenolic components, has the following structure.

3. Results and discussion

Fig. 5 shows the indices of reduction of the radical scavenging activity (% RSA) of the different extracts in function of their concentration. The graphs show that the radical scavenging activity strongly depends on the concentration – the higher the concentration, the lower the reduction of the absorbance of DPPH° and the higher the percentage of its reduction (% RSA). For the four extracts studied, it can be seen that at equal concentrations the radical scavenging ability of the tea extracts is higher than that of *Glycine max*, red wine and *Hibiscus sabdariffa*. These results confirm the correlation between content of phenolic components and the radical scavenging activity which has been described in numerous studies (Huh et al., 2004; Makris et al.,2007; Angelov et al., 2008; Baydar et al., 2007). Based on these observations, the following order of the radical scavenging activity of the studied extracts is proposed:

$$\%\ \mathrm{RSA(Tea)} > \%\ \mathrm{RSA}\ \ (\textit{Glycine max}) > \%\ \mathrm{RSA}\ \ (\text{red wine}) > \%\ \mathrm{RSA}\ \ (\textit{Hibiscus sabdariffa})$$

The reaction between the DPPH° radicals and the antioxidant compounds in the studied extracts is due to the presence of the electron on the nitrogen atom (Fig. 4). Because of the delocalisation of this electron, DPPH° is relatively stable in

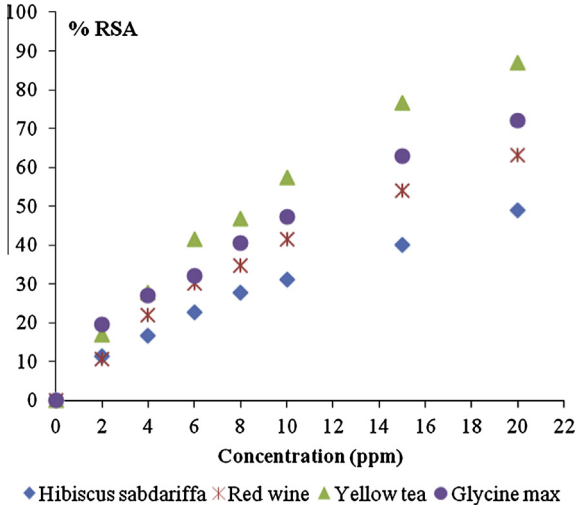
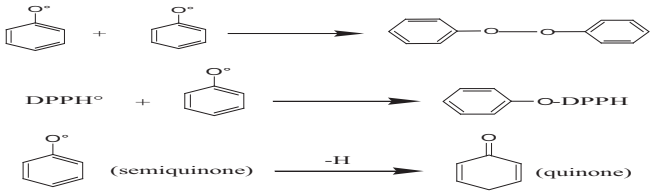


Figure 5 Indices of reduction of the radical scavenging activity (% RSA) in function of the concentration.

its monomer form at standard temperature. The delocalisation is also responsible for the characteristic blue colour of the DPPH°.The effectiveness of an antioxidant is determined by measuring the reduction of the blue colour caused by the recombination of the DPPH radicals.The capture of free radicals by antioxidants can be attributed to two mechanisms: (i) the liberation of the hydrogen atom of the hydroxyl group (rapid kinetic of certain acids and phenolic derivates): (ii) the liberation of an electron (slow kinetic of the glycolysed derivates and the anthocyanins) (Nanjo et al., 1996). In the case of phenolic compounds, the principal mechanism is the capture of free radicals through the capture of the H atom on the DPPH° to create a stable DPPHH molecule (Molyneux, 2004; Sanchez-Moreno et al., 1998).

Several reaction paths lead to the formation of more or less stable structures:



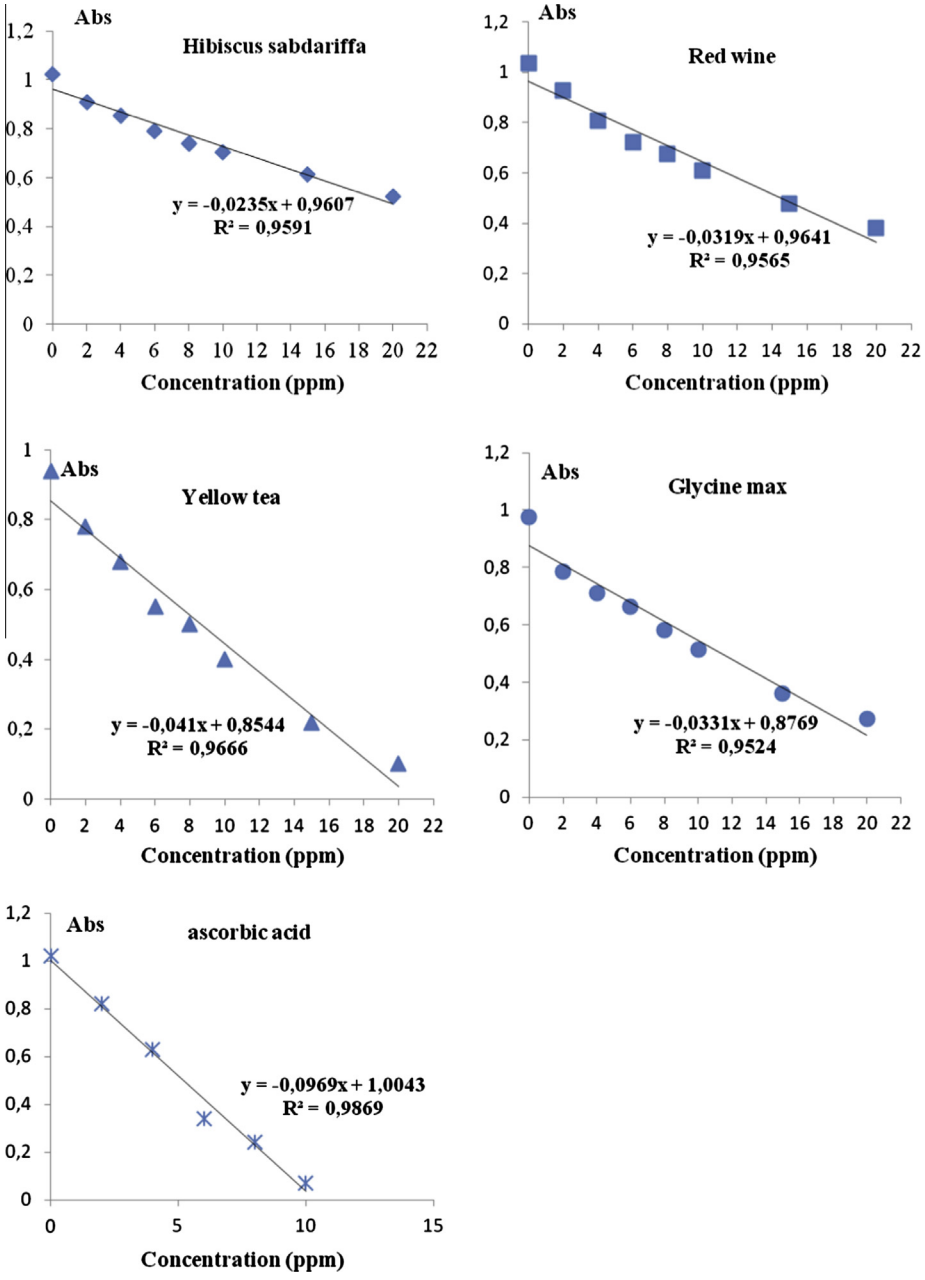


Figure 6 Absorbance in function of the concentration (ppm) for *Hibiscus*, *Glycine max*, tea, red wine and ascorbic acid.

Table 1 Colouring intensity of the tested extracts and of ascorbic acid.

Samples	I Pente I	IC_{50} (μM)
Ascorbic acid	0.097	4.62
Yellow tea	0.047	2.24
Glycine max	0.033	1.57
Red wine	0.031	1.47
Hibiscus sabdariffa	0.023	1.10

The radical scavenging capacity (capacity to bind free radicals, and thereby halt the chain reaction) cannot be measured directly, by only observing the effect of the reactivity. Several factors affect the antioxidant potential and the reduction kinetics, notable among them the phenolic profile (Molyneux, 2004).

In the present test subjects, it could be concluded that the resulting order can be explained by the structure of the phenolic compounds. Studies of the relationship between the chemical structure of phenolic compounds and their capacity to scavenge free radicals have shown that the scavenging activity depends on the number, position and nature of the substitutes of the B and C cycles and on the degree of polymerisation (Tabart et al., 2009; Nanjo et al., 1996; Karamac et al., 2005; Pannala et al., 2001).

These parameters are also linked to the polarity of the components. The higher activity of the tea, compared to that of the other studied beverages, must therefore be due to its high content of epigallocatechin (Nanjo et al., 1996), which gives it a higher polarity, when compared to the flavonoids found in the *Glycine max*, red wine and *Hibiscus sabdariffa* extracts. The differences between these three can be explained by the nature of the substitutes on the B and C cycles (Figs. 1 and 3).

In order to determine the effectiveness of each extract, the IC_{50} of each was determined. The Fig. 6, present the change in the absorbance in function of the concentration (ppm) of each extract. The incline of each line enables the IC_{50} of each extract to be determined. Their values are listed in Table 1 and compared to those of ascorbic acid.

The present study shows that the extracts of tea, *Glycine max*, red wind and *Hibiscus sabdariffa* have a lower antioxidant activity than ascorbic acid. These results confirm those of earlier studies. The correlation coefficient between the content of polyphenols and the antioxidant effect is highly significant ($R2 > 0.95$) for all the tested extracts. This indicates that over 95% of the antioxidant capacity of the extracts is due to the phenolic components. These results are in agreement with those reported by Wong et al., 2006; Turkmen et al., 2007; Wojdylo et al., 2007; and Djeridane et al., 2006 which demonstrated a positive correlation between the total content of phenolic compounds and the antioxidant activity.

4. Conclusion

The results of the tests with the DPPH° of the extracts of tea, red wine, *Glycine max* and *Hibiscus sabdariffa* show that these extracts have a considerable radical scavenging activity, whereby the activity of the tea is higher than those *Glycine max*, red wine and *Hibiscus sabdariffa*. It should however be noted that *Glycine max* has a slightly higher antioxidant activity than that of red wine, which is known for its health benefits. These results are directly linked to the quantitative and/or qualitative diversity of the compounds found in the extracts.

Although the study showed that the activity of *Glycine max* and *Hibiscus sadbariffa* is lower than that of ascorbic acid (vitamin C) and of tea, it provided an appraisal of the antioxidant potential of extracts of these two plants. The study therefore contributes to the evaluation of these two plants and provides evidence for the effect of a diet which is rich in *Glycine max* and *Hibiscus sabdariffa* on the prevention of cellular damage, as they provide antioxidants. Specific studies of the isolated compounds of these plants would be assisting to determine the effectiveness of each for scavenging free radicals.

Acknowledgement

The effort done by staff members of Chemistry Department, Faculty of Science, University of Sciences and Technology of Masuku (Franceville) Gabon, to contribute in this work is acknowledged.

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