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Research Article

Determination of total phenolic content and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) Calyx ethanolic extract

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Abstract

Background: Medicinal plants contain physiologically active ingredients that over the years have been exploited in traditional medicine for the treatment of various ailments. Objectives: This study was undertaken to investigate the total phenolic content and antioxidant capacity of Roselle (*Hibiscus sabdariffa*) calyx ethanolic extract. Methods: The total phenolic content was estimated by Folin Ciocalteau method using Gallic acid as standard while the antioxidant capacity was determined based on the plant extract to scavenge DPPH radical. Results: The total phenolic content was found to be 41.07 mg Gallic acid equivalent /g. The extract exhibited a notable dose dependent inhibition of the DPPH activity. At a concentration 250 µg/ml, *Hibiscus sabdariffa* calyx extract scavenged 86% of DPPH radical whereas 125 and 50 µg/ml caused 53% and 23% DPPH inhibition respectively, and a very mild inhibition was produced at a concentration 5 µg/ml. Conclusion: It can be concluded that *Hibiscus sabdariffa* calyx could be a potential source of antioxidant principles.

Keywords: Phenolic compounds; Antioxidant activity; Reactive Oxygen Species; *Hibiscus sabdariffa* calyx.

INTRODUCTION

Oxygen free radicals, classified under the more general term of Reactive Oxygen Species (ROS) which includes non-radical species such as hydrogen peroxide, are highly reactive transient chemical species formed in all tissues during normal aerobic cellular metabolism, with the potential to initiate damage to the various intracellular components (nucleic acids, lipids, proteins) on which normal cell functioning depends. Free radicals provoked by various environmental chemicals as well as endogenous metabolism are involved in a number of diseases like tumors, inflammation, shock, atherosclerosis, diabetes, infertility, gastric mucosal injury, brain dysfunction, cancer and ischemia due to the oxidative damage to DNA, lipids and proteins, which can result in failure of cellular functions. Free radicals and ROS are controlled in biological systems by some enzymes possessing antioxidant activities such as superoxide dismutase and peroxidase (Fattman *et al*., 2003; Kasai *et al*., 2000).

The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds. Phenolic antioxidants are reported to quench oxygen-derived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical and the antioxidant activity of phenolics in several systems has indicated that they were as active as Buthylated hydroxyaniscle (BHA) or Buthylated hudroxytoluane (BHT) of the

same functional group (Mammadov *et al*., 2011).

An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 2, 2 diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al.*, 2002).

Roselle (*Hibiscus sabdariffa*) is an edible plant used in various applications including foods. Among them, the most popular are the fleshy red calyces used for making wine, juice, jam, syrup, pudding, cakes, ice cream or herbal tea. Roselle flower and calyces is also known for its antiseptic, diuretic, antioxidant and antimutagenic properties (Salleh *et al.*, 2002). Roselle is an important source of vitamins, minerals and bioactive compounds such as organic acids, phytosterols and polyphenols, some of them with antioxidant properties. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin and their respective glycosides; protocatechuic acid, eugenol and sterols like β-sitoesterol and ergoesterol (Ali-Bradeldin *et al*., 2005). Roselle calyx extract is a good source of antioxidants especially anthocyanins (Ajiboye *et al*., 2011). Therefore, this study aims to investigate the total phenolic content and antioxidant capacity of Roselle (*Hibiscus sabdariffa*) calyx ethanolic extract.

MATERIALS AND METHODS

MATERIALS

Chemicals and reagents

2, 2 diphenyl-2-picryl hydrazyl (DPPH), Gallic acid and quercetin were purchased from Sigma –Aldrich company (UK). Folin Ciocalteau reagent was purchased from Merck Company (Germany).

Plant material

The dried calyces of *Hibiscus sabdariffa* were purchased from the local market in Wad-Medani, Sudan. The plant material was identified by the Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan.

METHODS

Extraction of plant material

One hundred grams of coarsely powdered calyces of *Hibiscus sabdariffa* were extracted by maceration using ethanol (70%) in a conical flask for 72 hours in dark, filtered and evaporated by a rotary evaporator at 40 ^{o}C. The resulting solution was freeze dried and kept in a refrigerator until use.

Determination of total phenolic content in *Hibiscus sabdariffa* calyx ethanolic extract

The total phenol content in the ethanolic extract of *Hibiscus sabdariffa* calyx was determined with Folin Ciocalteau reagent by the method described by Chinedu *et al*., (2011) .The crude extract (50 mg) was mixed with Folin Ciocalteau reagent (1ml) and deionized water (7.5 ml). The mixture was kept at room temperature for 5 minutes then 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 minutes at room temperature. After incubation, the absorbencies against the reagent blank were determined at 760 nm using UV/visible spectrophotometer .The total phenolic content of the plant was expressed as mg/g Gallic acid equivalent. All samples were analyzed in triplicates.

Antioxidant activity of *Hibiscus sabdariffa* calyx ethanolic extract

Sample stock solution (1 mg/ml) was diluted to final concentrations 250, 125, 50, 10 and 5 µg/ml in ethanol. One ml of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in ethanol solution was added to a 2.5 ml solution of the different concentrations of the extract and allowed to react at room temperature for 30 minutes. The absorbance of the result in mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below:

$$AA\% = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as control. Stock solution (1 mg/ml) of quercetin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in ethanol used as positive control (Mensor *et al*., 2001). All experiments were done in triplicates.

A freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 518 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of test compound to act as free radical scavenger. Furthermore, the "efficient concentration" or EC_{50} value, which is the concentration of antioxidant that causes 50% loss of the DPPH activity (colour), was also used to assess the antioxidant activity of the plant extract compared to the standard drug. The higher the antioxidant activity, the lower is the value of EC_{50} (Molyneux, 2004).

RESULTS

Total phenolic content:

The total phenolic content of *Hibiscus sabdariffa* calyx was found to be 41.07 mg Gallic acid /g (Table 1).

Table 1. Total phenolic content of *Hibiscus sabdariffa* calyx ethanolic extract

Type of analysis	Concentration in mg Gallic acid /g sample
Total phenolic content	41.07 mg Gallic acid /g.

Antioxidant activity

In this quantitative assay, the extract exhibited a notable dose-dependent inhibition of the DPPH activity. At a concentration 250 µg/ml, *Hibiscus sabdariffa* calyx ethanolic extract scavenged 86% of DPPH radicals whereas 125 and 50 µg/ml caused 53% and 23% DPPH inhibition, respectively. On the other hand, very mild inhibition was produced at by the concentration 5 µg/ml. The EC_{50} of the extract was found to be 22%. Meanwhile, the standard antioxidant agent, quercetin, produced 89.7% scavenging activity at a dose 250 µg/ml, followed by 85.8 % at 125 µg/ml, 62.1 % and 37.5% and 25% at doses of 50, 10 and 5 µg/ml respectively (Table 3 and Figure 2).

Table 2. DPPH scavenging activity of *Hibiscus sabdariffa* calyx ethanolic extract

Concentration µg/ml	Scavenging activity %
5	2
10	11
50	23
125	53
250	86

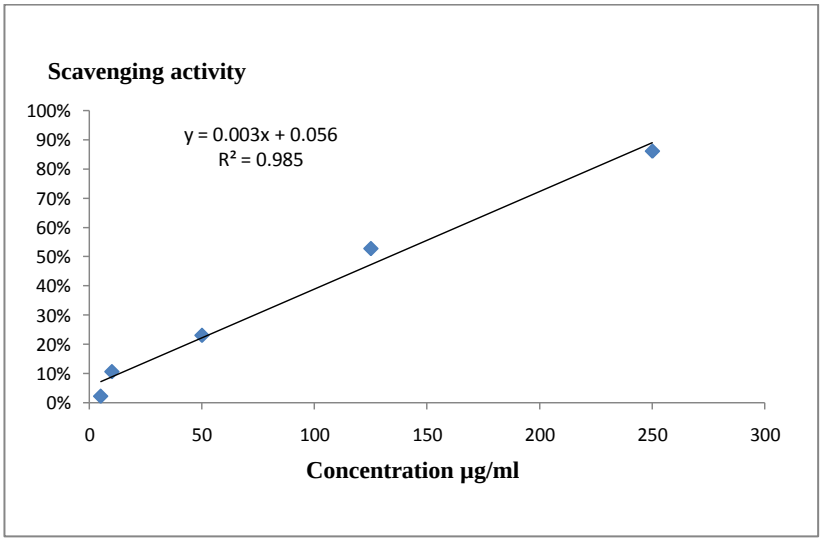

Figure 1. DPPH scavenging activity of *Hibiscus sabdariffa* calyx ethanolic extract

Table 3. DPPH scavenging activity of quercetin

Concentration µg/ml	Scavenging activity %
5	25
10	37.5
50	62.1
125	85.8
250	89.7

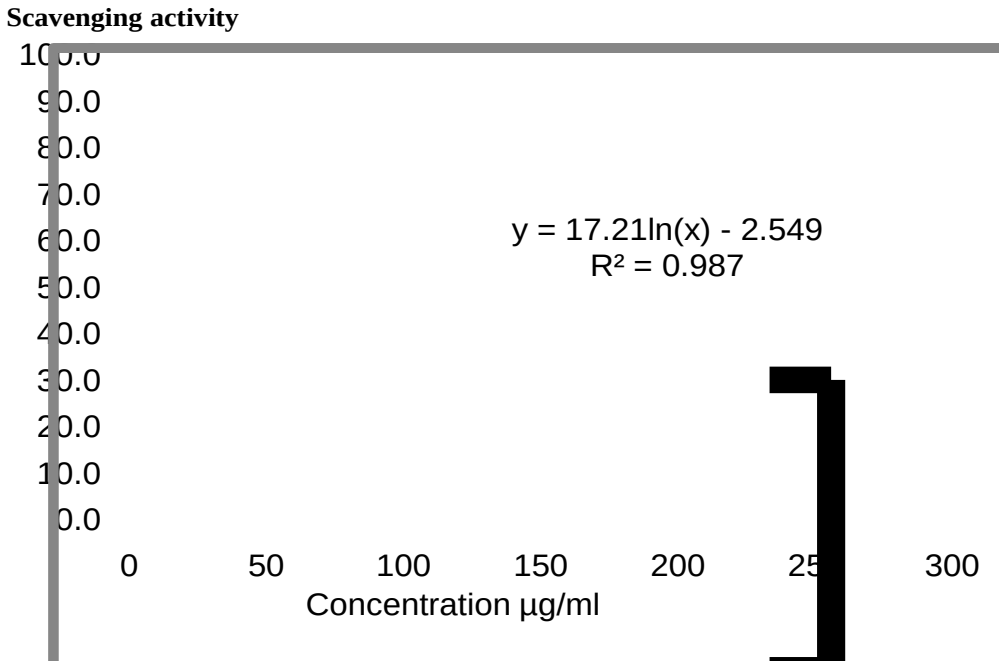

Figure 2. DPPH scavenging activity of Quercetin

DISCUSSION

The total phenolic content of *Hibiscus sabdariffa* calyx was found to be 41.07 mg Gallic acid /g (Table 1) similar to those reported by other researchers (Enrico *et al*., 2007; Azza *et al*., 2011; Chinedu *et al*., 2011) who also ascribed the antioxidant activity to these compounds.

The 2, 2 diphenyl-2-picryl hydrazyl(DPPH) radical widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic or crude extracts of plants. DPPH is a relatively stable radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent (Mensor *et al*., 2001).

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 518 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by its hydrogen donating ability. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up (Chinedu *et al*., 2011).

Today, medicinal plants are showing tremendous promise for preventive intervention in the pathogenesis of many diseases, as well as in their treatment (Atawodi, 2005), especially the ROS-mediated diseases such as cancer (Mantle *et al.*, 2000) and ulcer (Repetto and Liesuy, 2002). The relationship between the antioxidant compounds in plants and their effectiveness in the treatment of these diseases have been previously described (Repetto and Liesuy, 2002; Sabu and Kuttan, 2002; Yang *et al.*, 2001; Manach *et al.*, 2004).

Hibiscus sabdariffa calyces are used in folk medicine against many complaints that include high blood pressure, refrigerant, antiseptic, aphrodisiac, astringent, demulcent, digestive, sedative, stomachic and tonic (Mahadevan and Pradep, 2009).

In fact, Wang *et al*. (2000) reported that, Hibiscus anthocyanins were able to quench the free radicals of DPPH and this antioxidant effect was also demonstrated by the ability of the anthocyanins to reduce the cytotoxicity induced by tert-butylhydroperoxide in rat primary hepatocytes and to attenuate hepatotoxicity in rats as well as to protect DNA damage (Lazze *et al*., 2003) and cytotoxicity (Tseng *et al*., 1996).

In view of the established strong antioxidant and antilipid peroxidation actions of *Hibiscus sabdariffa* extracts and the compounds they contain (Wang *et al*., 2000; Suboh *et al.,* 2004), and because many diseases and conditions (for example, diabetes and aging) are thought to involve lipid peroxidation and the generation of free radicals (Poon *et al*., 2004; Vincent *et al*.,2004), the anthocyanins and Hibiscus protocatechuic acid may potentially be useful in ameliorating or preventing these diseases and conditions.

The effectiveness of *Hibiscus sabdariffa* calyx in termination and/or scavenging free radicals was reported by so many research workers (Chinedu *et al*., 2011; Alaa, 2012; Liuqing *et al*., 2012) who documented that the antioxidant activity of *Hibiscus sabdariffa* calyx could be attributed to the presence of phenolic compounds. Moreover, it was reported that the extract of Roselle was found to be very high in ascorbic acid content or ascorbate, which is a well-known natural antioxidant and excellent reducing agent (Buettner and Jurkiewicz , 1996; Falade *et al*., 2005; Prenesti *et al*., 2007;).

The antioxidant activity of various *Hibiscus sabdariffa* extracts proved to protect induced hepatotoxicity (Liu *et al*., 2002), DNA damage (Lazze *et al*., 2003) and cytotoxicity (Tseng *et al*., 1996).

It can be concluded that *Hibiscus sabdariffa* calyx could be a potential source of antioxidant principles.

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