Antioxidant Activity and Total phenol and flavonoid contents of *Croton zambesicus* Muell. Arg seed extract and fractions

Abstract

The present study was designed to investigate the antioxidant activity of *Croton zambesicus* Muell. Arg seed extract and fractions (Family: *Euphorbiaceae*). The antioxidant activities were conducted via DPPH free radical scavenging assay. Potential antioxidant activity was presented by methanolic extract. *C. zambesicus* showed high DPPH value (83.21 ± 0.05). Crude extract was fractionated using separation funnel, the five fractions were obtained are hexane, chloroform, ethyl acetate, n-butanol and water which represented antioxidant activities equal to 46.2 ± 0.1349, 45.1 ± 0.0116, 49.9 ± 0.0337, 40.45 ± 0.2290 and 62.7 ± 0.0006 respectively. The results of total phenol and flavonoid contents showed high value for hexane extract (31.846 mg QE/g) followed by 23.692, 23.538, 23.538 and 22.615 mg QE/g for chloroform, ethyl acetate, n-butanol and water respectively. While the high value for total Phenol represented by ethyl acetate (0.173 mg GAE/g). The study gives rise to antioxidant property of studied plants, and showed interesting correlation with antioxidant activities and their phytochemical constituents.

Key words: *Croton zambesicus*, antioxidant activity, phenol and flavonoid contents, separation funnel.

INTRODUCTION

Sudan is the largest country in Africa with a diverse flora. Most of the Sudanese people in rural areas rely on flkoloric medicine for the remedy of many infectious diseases. Sudanese traditional medicine is characterized by a unique combination of knowledge and practices of Arabic, Islamic and African culture (Ietidal *et al*., 2010).

Plants are the largest drug stores ever known on Earth, by producing endless bioactive chemical compounds which have direct effects on animal and human health (Abdallah, 2011). Today, most of the modern drugs (synthetic or semi-synthetic) are initially produced from natural products such as medicinal plants prescribed in the ancient traditional medicine (Sukanya *et al.*, 2009).

Recently, the interest in medicinal plants is growing, since many plant species have been recognized to have medicinal benefits and positive impact on human health, such as anti-inflammatory, antibacterial, hypolidemic, anti-carcinogenic, anti-oxidant and many others (Cai *et al.*, 2004). Though, most of the Sudanese people rely on medicinal plants as a primary health care system instead of the expensive modern medicine especially in rural area. WHO (2001) reported that there are more than 2000 medicinal plants in use, which are recorded in "The Sudan Atlas of Medicinal Plants", but it is believed that the number of medicinal plants that in current use may be much more than that reported.

Reactive oxygen species, such as single oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive toxic molecules, which are generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals (Saeed et al., 2012).

The plant *Croton zambesicus* Muell. Arg (Syn. Name: *C. amabilis* Muell. Arg.) (Family euphorbiaceae). It is a species of widely spread in tropical Africa (Fig. 1). The root used for menstrual pain (El- Hamidi, 1970) DNA as aperients (Ngadjui *et al*., 1999). The root is also used in Sudan as anti malarial and anti diabetic (El- Hamidi, 1970; Okokon and Nwafor, 2009).Also it used as anti diabetic and malarial remedy in Nigeria (Okokon *et al*., 2005; Okokon *et al*., 2006). The seed decoction is commonly used in Sudan to treat cough, malaria and to relieve menstrual pain (El Kamali and Khalid, 1996), also it used by women for hair elongation. Hence, there is need to investigate the antioxidant activity of seed extract and its fractions as well as their phytochemical correlation with antioxidant activity.

Materials and methods

Plant materials

Balanites aegyptiaca and Croton zambesicus were collected from local market in Omdurman, while *Zizyphus spina-christi* plant was collected from the home garden and authenticated at Botany Department, Faculty of Séance and Technology, Omdurman Islamic University.

Figure (1): Fruit of *Croton zambesicus*

Preparation of crude plant extracts

The plant material was air dried and ground into coarse powder using mortar and pestle. Two hundred grams were soaked in methanol for three days in a shaker then filtered using Whatman No. 3 filter paper. The filtrates were evaporated to dryness using a rotatory evaporator and weighed.

Fractionation procedure

The plant material fractionated using separation funnel into five solutions according to the degree of polarity. The crude extract was fractionated using liquid- liquid extraction methodology, which was carried by dissolving the sample in dist. H2O then they were partitioned between n-hexane chloroform, ethyl acetate, and n-butanol using separation funnel apparatus.

Antioxidant activity

In order to evaluate the antioxidant potentials, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and iron chelating techniques were used. The DPPH radical scavenging was determined according to the method of Shimada *et al*. (1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µml. The test samples were dissolved in DMSO (Dimethyl sulfoxide) while DPPH

was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multi plate reader Spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and Propyl Gallate (PG). All tests and analysis were run in triplicate.

Total Flavonoid content

Total flavonoid content of the extract was estimated using the aluminum chloride ($AlCl_3$) colorimetric method reported by Chang et al. (2006), 5 mg of the extract was dissolved in 1 ml of the test solvent to obtained the analyte. Calibration curve was prepared using, 1 mg of quercetin dissolved in 1 ml of absolute methanol. Seven different concentrations of the resulting quercetin solution were prepared serially in 2-folds (0.015625 -1 mg/ml). 100 µl of each extract or quercetin concentration was transferred into the cuvettes in triplicates.100 µl of 2% aluminium chloride ($AlCl_3$) was added to each well. The plate was thoroughly shaken and incubated at room temperature for 20 minutes. After the incubation, absorbance was read at a wavelength of 415 nm using AT1 UNICAM UV/VIS spectrometer (UV4 coupled to Vision V3.40 computer software). From the absorbance readings, the total flavonoid content of each extract was calculated from the regression equation of the quercetin standard curve and expressed as quercetin equivalents (QE), and the result was expressed as mg quercetin equivalent per g dry weight.

Total phenolic content

The total phenolic content of the extract was determined by the Folin-Ciocalteu method (Kaur and Kapoor, 2002). Briefly, 200 L of crude extract (1 mg/mL) was made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per g dry weight.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program (2007).

Results and discussion

The antioxidant activity can be expressed in various ways and one of the most common ways is to express by referring it to a common reference standard. The result showed that *Croton zambesicus* indicated a large spectrum antioxidant activities (83.21 ± 0.05), Table (1). The antioxidant activity for *Croton zambesicus* agree with what was reported by Manal et.,al. (2015) who proved that the ethanolic extract was able to reduce the DPPH free radicals (89%). High percentage of antioxidant activity is attributed to the high presence of phenol and flavonoid contents which was in accordance with literature report that indicated high correlation between antioxidant activity and total phenolic content (Ofentse *et al*., 2015).

Table (1): Antioxidant activity of plant extract using DPPH

Sample	%RSA ±SD (DPPH)
Croton zambesicus	83.21 ± 0.05
Propyl galate	93+0.01

RSA = Radicals scavenging activity DPPH= 2, 2, Diphenyl -1- Picrylhydrazyl**.**

The *Croton zambesicus* extract which showed high potential of scavenging activity fractionated using separation funnel apparatus. The reduction power decreases inversely according to the polarity of extraction solvent (Dejian *et al*., 2005) and the capacity of reduction of a compound may serve as a significant indicator of its antioxidant potential (Hassan *et al*., 2016).

Corton zambesicus fractions of seed were able to inhibit the DPPH activity, the fractions were showed varied potentials (Table.2). The five fractions were obtained are hexane, chloroform, ethyl acetate, n-butanol and water which represented antioxidant activities equal to 46.2 ± 0.1349, 45.1 ± 0.0116, 49.9 ± 0.0337, 40.45 ± 0.2290 and 62.7 ± 0.0006 respectively. Water fraction was mainly the most active maybe due the nature of water extract which have high polarity represented active constituents. The result showed low values when compared to that study obtained by Mohamed et al., (2016). Azaizah et al., (2003) stated that medicinal plants with bioactive compounds may act individually, additively or synergistically to improve health.

The result clearly indicated that the plant had high antioxidant effect for crude extract which was attributed to additively effects of the compounds, while synergistically effect showed in plant fractions. However, this is disagreement with what was reported by Mohamed et al., (2016) who tested fruit plant.

Total phenolic content in Table (3) has a good positive correlation with DPPH radical scavenging. This was in accordance with literature report which reported high correlation between antioxidant activity and total phenolic content (Ofentse *et al*., 2015).

The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Plant flavonoids are currently have risen interest owning to their supposed properties in promoting health (anti-oxidants) (Rauha *et al*., 2000); Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages (Rasouli et., al.2017). The results of total flavonoid contents showed high values for hexane fraction (31.846 mg QE /g) followed by 23.692, 23.538, 23.538 and 22.615 QE mg/g for chloroform, ethyl acetate, n-butanol and water fractions respectively. While the high value for total Phenol represented by ethyl acetate fraction (0.173 mgGAE/g). Phenolic compounds are play an important role in the defense of plants against pathogens, diseases, parasites, and predators (Bhattacharyya et al., 2014). Furthermore, they affect in a number of physiological mechanisms such as antioxidant activity. The study gives rise to antioxidant property of studied plant, and showed interesting correlation with antioxidant activities and their phytochemical constituents.

The only study which conducted in seeds plant fractions was reported by Hiba and Elamin (2017) who represented the traditional uses of C. zambesicus seeds and screened antioxidant activities using colorimetric screening. This is a clear indication for the first time achievement of results which were not recorded in any previous work in the available literature using correlation between antioxidant activity and their reservoir of flavonoid and phenolic contents.

Table (2): Antioxidant activity of *Croton zambesicus* fractions

Sample	%RSA ±SD (DPPH)
Water	62.7 ± 0.0006
Ethyl acetate	49.9 ± 0.0337
N-butanol	40.45 ± 0.2290
Hexane	46.2 ± 0.1349
Chloroform	45.1 ± 0.0116

Table (3): Total Phenolic and flavonoid contents of *Croton zambesicus* extracts

Fraction	Flavonoid mg QE/g of Extract	Total Phenol mg GAE/g extract
Water	22.615	0.008
Ethyl acetate	23.615	0.072
N-butanol	23.579	0.173
Hexane	31.846	-0.015
Chloroform	23.692	-0.253

Conclusion

Croton zambesicus that used as folkloric medicine in Sudan possess high significant antioxidant activity and total phenol and flavonoid contents. hence, it might be involved as natural therapeutic and cosmetic agent.

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