Antioxidant activity of *Croton zambesicus* Muell. Arg seed extract

Abstract

The present study was designed to investigate the antioxidant activity of *Croton zambesicus* Muell. Arg seed extract and fractions (Family: *Euphorbiaceae*). The antioxidant property was assessed by testing for DPPH free radical scavenging activity. 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.

Key words: *Croton zambesicus*, antioxidant activity, , separation funnel.

INTRODUCTION

"Sudan is the largest country in Africa with a diverse flora. Most of the rural population rely on folkloric medicine to cure some diseases. Also the 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.

"Antioxidants are substances that can scavenge the free radicals which produced from the oxidation processes. Many of them can be generated naturally in plants. They have interest in research to develop cures for age-related diseases. Through various plant secondary metabolites, phenolic and flavonoid groups are abundant in almost all plant materials and believed to represent good antioxidant activity" (Maria et al., 2020). "The free radicals are highly reactive toxic molecules, such as single oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, which are produced normally in cells during metabolism. They involved in numerous sever 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), was distributed in tropical Africa (Fig. 1). The root used for menstrual pain" (El-Hamidi, 1970) and aperients (Ngadjui *et al*., 1999). "Also it used as anti malarial and anti diabetic in Sudan" (El- Hamidi, 1970; Okokon and Nwafor, 2009). "Moreover it used as anti diabetic and malarial remedy in Nigeria" (Okokon *et al*., 2005; Okokon *et al*., 2006). "In Sudan the seed decoction usually used to treat cough, malaria and to relieve menstrual pain (El Kamali and Khalid, 1996), also it used by women for hair elongation". This study is aimed to investigate the antioxidant activity of plant extract and compare it with their fractions.

Materials and methods

Plant materials

Croton zambesicus seeds purchasedfrom local market in Omdurman, 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. H_2O 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".

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 fractionated using separation funnel apparatus. The reduction force decreases inversely according to the polarity of solvent which is used in the extraction method (Dejian *et al*., 2005) and the reduction ability of a compound may act 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 may be 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. However, this is disagree with what was reported by Mohamed et al., (2016) who tested fruit plant.

The only study which conducted in seed fractions was reported by Hiba and Elamin (2017) who reported the traditional uses of C. zambesicus seeds and detected the constituents by phytochemical screening test. .

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

Conclusion

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

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