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In vitro antioxidant activity and phytochemical screening of *Croton zambesicus*

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Abstract
The present study was designed to investigate the antioxidant activity of *C. zambesicus* fruits and whole plant. Phytochemical study was piloted to detect the bioactive compounds, which have been responsible for the biological activities. The antioxidant activities were conducted via DPPH radical scavenging assay. Potential antioxidant activity was presented by ethanol crude extract was motivated to evaluate the fractions of hexane, chloroform, ethyl acetate, n-butanol and water, the radical scavenging activities of whole plant were found to be 72 ± 0.03, 79 ± 0.02, 80 ± 0.05, 89 ± 0.01, 85 ± 0.01, 77.3 ± 0.05 respectively, and the fruit extracts were found to be 81 ± 0.03, 41.4 ± 0.01, 51 ± 0.04, 85 ± 0.01, 88 ± 0.02, 77.3 ± 0.01 respectively. The results of phytochemical screening showed the presence of Flavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpene, Phytosterol, Anthraquinones and Carbohydrates. This study give rise to antioxidant property of studied plant, and showed interesting correlation with the phytochemical constituents and biological activities.

Keywords: *In vitro*, *Croton zambesicus*, Antioxidant, Phytochemical Screening, Radical Scavenging.

1. Introduction
Medicinal plants are still invaluable source of safe, less toxic, cheap, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against the disabling diseases (Amaral *et al*., 2006; Koko *et al*., 2008) [3, 15].

Antioxidants and free radical scavengers (Colic and Pavelic, 2000) [5]. Plants are susceptible to damage caused by active oxygen and thus develop numerous antioxidant defence system resulting in formation of numerous potent antioxidants. In simple words "Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain disastrous enemies (diseases) such as arterial and cardiac diseases, arthritis, cataracts and also premature ageing along with several chronic diseases." Plants contain certain chemicals such as carotenoids, flavonoids, bioflavonoids, phenols, phytosterols etc. that possess antioxidative properties. Since reactive oxygen radicals play an important role in carcinogenesis and other human disease states, antioxidants present in plants have received considerable attention as cancer chemopreventive agents (Lee *et al*, 1998) [16].

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997) [28]. Oxidative stress refers to the physiological condition at which the capacity of the endogenous antioxidant system fails to cope with the damaging effects of free radicals. Strong experimental evidences have been established about the oxidative stress theory of Alzheimer's disease pathogenesis where oxidative damage plays a major role in neurological degeneration (Mariani *et al*., 2005) [5].

All plants produce chemical constituents, part of their normal metabolic activities (Tyler *et al*., 1981, Rosenthal *et al*., 1979) [31, 26]. These, can be divided into primary metabolites, such as sugars, amino acids, nucleotides and fats, found in all plants, and secondary metabolites which have no obvious function in a plant's primary metabolism as well as in growth, photosynthesis, or other ''primary'' functions of the plant cell. They may possess an ecological role, as pollinator attractants, represent chemical adaptations to environmental

stresses, or to be responsible for the chemical defence of the plant against microorganisms, insects and higher predators (Harborne *et al*., 1982, Trease and Evans, 1989) [12, 29]. The plant *Croton zambesicus* Muell. Arg (Syn. Name: *C. amabilis* Muell. Arg.) (Family euphorbiaceae). It is a species of widely spread in tropical Africa. The root used for menstrual pain (El- Hamidi, 1970) [8] dna as aperients (Ngadjui *et al*., 1999) [19]. The root is also used in some regions of Nigeria as anti malarial and antidiabetic (Okokon and Nwafor, 2009) [23]. The leaf decoction is used in Benin as a wash for fevers, dysentery, convulsions, antihypertensive and as antimicrobial for urinary infections (Adjanohoun *et al*., 1989) and in parts of Nigeria as antidiabetic and malarial remedy (Okokon *et al*., 2005; Okokon *et al*., 2006) [24, 22]. The seed decoction is commonly used to treat cough, malaria and to relieve menstrual pain (El Kamali and Khalid, 1996) [9]. Hence, there is need to investigate the antioxidant activity and Phytochemical screening of fruits and whole plant extracts.

Material and Methods

Plant materials

Croton zambesicus fruits and whole plant were collected from (Khartoum) Central Sudan during the period of January to February 2016. The plant was identified and authenticated by the researcher Dr. Haider Abdelgadir, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). Khartoum, Sudan. Fruits and whole plant of *C. zambesicus* were air-dried, under the shade and pulverized and stored prior to extraction. Shade with good ventilation and then ground finely in a mill and kept in the herbarium until their uses for extract preparation.

Preparation of crude extract

Extraction were carried out for the fruits and whole plant of *C. zambesicus* by using overnight maceration techniques according to the method described by Harbone (1984) [11]. About 50 g of the dried powder from *C. zambesicus* fruits and whole plant were macerated in 250 ml of ethanol for 3 h at room temperature. Occasional shaking for 24 h was performed and, the supernatant were decanted. After this, the supernatant were filtered by using Clevenger's apparatus. The extracts were kept in freeze dryer for 48 h, (Virtis, USA) until they were completely dried. The extracts were kept and stored at 4 °C until required needed for analysis. The yield a percentage was calculated as follows:

Percentage = (weigh of extract/weigh of sample) X 100.

Fractionation

The crude extracts were fractionated using liquid- liquid extraction methodology, which were carried by dissolving the samples in dist. H_2O then they were partitioned between n-hexane chloroform, ethyl acetate, and n-butanol using separation funnel apparatus.

DPPH Free Radical Scavenging Activity

The DPPH radical scavenging was determined according to the method of (Shimada *et al*., 1992) [27], with some modification. The test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37 ºC in 96-wells plate. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation decrease in absorbance was measured at 517nm using multiplet reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted triplicate.

Qualitative Phytochemical Evaluation

Phytochemical screening was conducted to determine the presence of natural products in the extract an obtained from the *C. zambesicus* fruit and whole plant.

The extracts obtained were subjected to following chemical tests for identification of various phytoconstituents using standard methods of (Trease and Evans, 1989; Odebiyi and Sofowora, 1978) [29, 20].

Test for Carbohydrates

Molisch's test

To the extract 1ml of the Molisch's reagent was added then along the walls of the test tube carefully concH_2SO_4 was added. Formation of a brown ring at the junction of the two liquids was observed.

Benedict 's test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Barfoed's test

To the extract in a test tube 1ml of Barfoed reagent was added and boiled on the water bath. The solution was observed for the colour change reaction.

Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Tannins (Ferric Chloride)

0.5ml of the extract was boiled with 10ml of Distilled water in a test tube and then, few drops of 0.1% Ferric Chloride solution was added and the reaction mixture was observed for blue, greenish black colour change.

Test for Saponins (Frothing Test)

0.5ml of the extract was added to 5ml of Distilled water in a test tube. The solution was shaken vigorously and observed for the stable persistent froth.

Test for Flavonoids

Three Different Tests Were Used for the Flavonoid Identification.

Alkaline reagent test

To 0.5ml of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

KOH test

To 0.5ml of extract was treated with few drops of alcoholic potassium hydroxide solution. Formation of intense yellow colour.

Test for Terpenoid and Steroids
Salkowski test was used to identification steroid and terpenoid.
To 0.5ml of each of the extract 2ml of chloroform was added and then 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids and steroids.

Test for alkaloids
Two different tests were used for the identification of alkaloids

Dragendroff's Test
Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Wagner's Test
Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for Anthraquinones (Borntragor's test)
To 0.5ml of the extract 5 - 10ml of dilute HCL was added and boiled on water bath for 10 minutes and filtered. Then the filtrate was extracted with carbon tetra chloride and the equal amount of ammonia was added. After shaking the reaction mixture was observed for the formation of pink – red colour in the ammonia layer.

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
C. zambesicus ethanolic extracts of fruit and whole plant were able to inhibit the DPPH activity with 81 ± 0.03 and 72 ± 0.03 respectively theirs fractions was showed varied potentials shown in Table (1). Ethyl acetate was mainly the most active maybe due the polarity of the active constituents.

Table 1: Antioxidant activity *C. zambesicus* extracts

sample	Antioxidant activity percentage ± SD						
	crude	hexane	Chloroform	Ethylactate	n-Butanol	Water residue	Control
fruit	81 ± 0.03	41.4 ± 0.01	51 ± 0.04	85 ± 0.01	88 ± 0.02	77.3 ± 0.01	87 ± 0.01
Whole plant	72 ± 0.03	79.5 ± 0.02	80 ± 0.05	89 ± 0.01	85 ± 0.01	77.3 ± 0.05	

Key: Control= Propyl galate, SD = Standerd divation.

Table (2 and 3) indicates the presence of pharmacologically useful classes of compounds (Flavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpene, Phytosterol, Anthraquinones) tested for. These secondary metabolites have been shown to have therapeutic activities in plants and function in a synergistic or antagonistic fashion for the treatment of diseases (Trease and Evans, 1996) [30].

Table 2: Phytochemical screening of *C. zambesicus* fruits

Family of compounds	Type of test	Inference				
		Hexane	Chloroform	Ethylactate	n-Butanol	Water residue
Carbohydrates	Molish's	+ve	+ve	+ve	+ve	+ve
	Bendect test	+ve	+ve	+ve	+ve	+ve
	Barafoids	+ve	+ve	+ve	+ve	+ve
Flavonoids	Lead acetate	+ve	+ve	+ve	+ve	+ve
	KOH	+ve	+ve	+ve	+ve	+ve
	Alkiline test	+ve	+ve	+ve	+ve	+ve
Saponins	Forth	-ve	+ve	-ve	-ve	+ve
Alkaloid	Dragendorff's	+ve	+ve	+ve	-ve	-ve
Tannins	FeCl3	+ve	+ve	+ve	+ve	+ve
Phenols	FeCl3	+ve	-ve	+ve	+ve	+ve
Triterpene	Salkowski	+ve	+ve	+ve	+ve	+ve
Phytosterol	Salkowski	+ve	+ve	+ve	+ve	+ve
Anthraquinones	Borntragor's	+ve	+ve	+ve	+ve	+ve

Key: +ve = Positive result -ve = Negative result.

Table 3: Phytochemical screening of *C. zambesicus* whole plant

Family of compounds	Type of test	Interference				
		hexane	Chloroform	Ethylactate	n-Butanol	Water residue
Carbohydrates	Molish's	+ve	+ve	+ve	+ve	+ve
	Bendect	+ve	+ve	+ve	+ve	+ve
	Barafoids	+ve	+ve	+ve	+ve	+ve
Flavonoids	Lead acetate	+ve	+ve	+ve	+ve	+ve
	KOH	+ve	+ve	+ve	+ve	+ve
	Alkiline test	+ve	+ve	+ve	+ve	+ve
Saponins	Forth	+ve	+ve	+ve	-ve	+ve
Alkaloid	Dragendorff's	+ve	+ve	+ve	-ve	-ve
Tannins	$FeCl_3$	+ve	+ve	+ve	+ve	+ve
Phenols	$FeCl_3$	-ve	-ve	+ve	+ve	+ve
Triterpene	Salkowski	+ve	+ve	+ve	+ve	+ve
Phytosterol	Salkowski	+ve	+ve	+ve	+ve	+ve
Anthraquinones	Borntragor's	+ve	+ve	+ve	-ve	-ve

Key: +ve = Positive result -ve = Negative result.

The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Plant phenolic compounds especially flavonoids are currently of growing 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 (Mark Percival, 1998) [18]. Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants (Tyler *et al*., 1988; Awosika, 1991; Ogunleye and Ibitoye, 2003) [32, 4, 21]. They act as binders and for treatment of diarrhea and dysentery (Dharmananda, 2003) [7]. Tannin also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic (Heslem, 1989) [13]. Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars (Aiyelaagbe and Osamudiamen, 2009) [2]. Plant steroids are known to be important for their cardiotonic, insecticidal and anti-microbial properties. They are also used innutrition, herbal medicine, cosmetics and they are routinely used in medicine because of their profound biological activities (Denwick, 2002) [6]. Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature and are reported to have anti-diabetic and anti-fungal properties (Finar, 1989; Trease and Evans, 1989; Kamel, 1991) [10, 29, 14].

Conclusion

All extracts of *C. zambesicus* fruits and Whole plant showed potent antioxidant activity. This might be attributed to the presence of phenols, flavonoids and tannins compounds in this plant. The effect of this plant bioactivities, and toxicological investigation and Further purification, need to be carried out also further phytochemical investigation of active constituents might be present is required.

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