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Research Article

Studies on the Antioxidant Potentials of *Croton spirale* (*Codiaeum variegatum var. spirale*) (L.) Leaf

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ABSTRACT

The present work was undertaken to investigate the antioxidant activity of *Croton spirale* (*Codiaeum variegatum var. spirale* (L.)). The leaves of *Croton spirale* were collected, dried, powdered and extracted in methanol and water. Quantitative analysis of phytochemicals (saponins, tannins, phytates, phenols, cardiac glycosides, flavonoids, oxalates, alkaloids), vitamins (A, B1, B2, B3, B6, B12, C and E), and minerals (zinc, iron and selenium) were determined using standard methods. The antioxidant scavenging activity was determined using DPPH (2,2-diphenyl-1-picryhydrazyl) method. Result showed that *Croton spirale* contains: flavonoids (13.16%),phenols (5.21%), tannins (128.32%), saponins (2.64%), phytates (3.00%), cardiac glycosides (2.00%), oxalates (0.024%), alkaloids (11.92%), vitamin A (3.12mg/100), vitamin B1 (7.01mg/100), vitamin B2 (5.15mg/100), vitamin B3 (0.585mg/100), vitamin B6 (0.534mg/100), vitamin B12 (23.53mg/100) vitamin C (6.231mg/100), vitamin E (1.5mg/100), zinc (1.906), iron (3.366), and selenium (2.786). These values are within FAO/WHO (2001) standard except for vitamin B6 which is slightly below the standard. The extract also has a high DPPH radical scavenging activity relative to standard ascorbic acid. These suggest that *Croton spirale* can be used in the management of oxidative stress and in prevention of diverse disease states such as allergies, inflammatory responses, microbial infections, and cancer.

Key words: *Croton spirale* leaf, Phytochemical constituents, DPPH scavenging activity, Reactive oxygen species (ROS), Antioxidant vitamins and minerals

INTRODUCTION

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen. They are chemical species that have an unpaired electron in their outer orbits. The unpaired electron gives the radical instability and it reacts easily with inorganic or organic chemicals. ROS including the superoxide anion ($\cdot O_2$), hydroxyl radical ($\cdot OH$), or their metabolites (hydrogen peroxide, H_2O_2; hypochlorous acid, HOCl) have been implicated in over 100 human clinical conditions (Cross *et al.,* 1987).

Exposure to hyperoxia (Freeman and Crapo, 1981) xenobiotics (Lieber, 1988), cigarette smoke or mineral dust (Janssen *et al.,* 1994) has been shown to cause oxygen radical production and tissue injury followed by both an acute and possibly a chronic inflammatory response. ROS are normally and continuously produced during aerobic respiration by the mitochondrial electron transport chain. Enzymatic processes such as those carried out by xanthine oxidase, cyclooxygenase, and lipoxygenase (Cross *et al.,* 1987) also result in the intracellular production of oxygen free radicals. Activated oxygen species are also generated as a consequence of exposure to ionizing radiations such as x-rays, ɤ-rays, and other forms of radiations (Cross *et al.,* 1987). These oxygen species damage cells by oxidation and interaction of enzymes, causes DNA base hydroxylation, nicking, and cross-linking and by peroxidation of polyunsaturated lipids in the plasma membrane which may then act as chemo-attractant leading to degenerative or pathological processes, such as ageing, cancers, coronary heart diseases, atherosclerosis, cataracts and inflammations (Cross *et al.,* 1987).

Although excessive oxygen radical production is a normal consequence of aerobic life, all aerobic organisms from bacterial to humans have developed mechanisms to detoxify ROS. Many of these mechanisms have remained highly conserved owing to their vital importance to the survival of the organism. These biochemical defenses

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include chemical antioxidants such as vitamins, minerals, and phytochemicals (Harris *et al.,* 1992), as well as the antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Harris *et al.,* 1992). These antioxidants function by interfering with the oxidative processes by acting as electron donors (Harris *et al.,* 1992).

The natural antioxidant mechanisms may be insufficient in variety of conditions; hence dietary intake of antioxidant compounds is therefore necessary. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has consequently been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods (fruits and vegetables) and incidence of human diseases. Plant-based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature (Cross *et al.,* 1987). These facts have inspired a wide-spread plant screening for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent these diseases (Akinmoladun *et al.,* 2007). Extracts (or infusions) of the different parts of the plants of several species of the genus, *Croton*such as *C. lechleri, C. palanostigma, C. draconoides and C. urucurana* are traditional remedies well known for their healing powers (Moudipa *et al.,* 2005). Freeze-dried leaf decoction of *C. variegatum* is taken as tea by Filipinos (Gertrude, 2006). Drinking of crushed leaves cures diarrhea. These characteristics can be attributed to the antioxidant property of these plants.

Consequently, the purpose of the present work is to study the antioxidant activity of *Croton spirale* leaf. This is done through investigating total phytochemicals (saponins, tannins, phytates, phenols, cardiac glycosides, flavonoids, oxalates, alkaloids), vitamins (A, B1, B2, B3, B6, B12, C, E), and minerals (zinc, iron, selenium) content of the plant, *Croton spirale*. DPPH scavenging activity and reducing power of the plant extract was also determined.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Sample

The leaves of *Croton spirale* (family of Euphorbiaceae) were collected from Agu-Awka, Anambra state, Nigeria during the dry season. The cleaned and non-infected leaves were spread, dried and powdered using a "Corona" manual grinder. About 300g of leaves powder was soaked in methanol and distilled water (4:1) for 72hours. The mixture was filtered using "Whatmann" filter paper and then evaporated to dryness under vacuum. The extract was stored in a cool place until use.

Determination of total phytochemicals

Total flavonoids were determined using the method described by Boham and Kocipai (1994). Total phenols were analyzed using spectrophotometric method. Polyphenols were determined using Follins Dennis titrating method as described by Pearson (1974). Other phyto constituents were determined using standard procedures (Harborne, 1993; Obadoni and Ochuka, 2002). In addition, the extract of *Croton spirale* was tested for different vitamins using standard procedures described by Kirk and Sowyer (1991).

Methods for the Metals Analysis

Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrometer according to the method of APHA 1995 (American Public Health Association).

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams *et al.* (1995) with slight modifications.

To one ml of different concentrations of the extract or reference was added one ml of 0.3mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30mins after which the absorbance was measured at 517nm against a DPPH control containing only one ml of methanol in place of the extract. The radical scavenging activity was then calculated using the formula;

$$\%\text{inhibition} = [(Ao - Ac)/Ao] \times 100$$

Where: Ao = Absorbance without extract, Ac = Absorbance with extract. All experiments were done in duplicate and the results averaged and reported.

RESULTS AND DISCUSSION

The aqueous methanolic extract of *C. spirale* yielded a dark-greenish paste after concentration in vacuo. Tables 1, 2 and 3 represent the results obtained after the qualitative phytochemicals, vitamins and minerals analyses of the leaf extract of *Croton spirale.* Figure 1 shows the DPPH scavenging activity of the leave extract relative to standard ascorbic acid.

The detrimental effects of damage on biological cells by reactive oxygen species, ROS calls for a consolidated effort to combat them. This is the driving force in evaluating experimentally, the antioxidant potential of *Croton spirale* through determination of its total phytochemicals, vitamins and minerals.

The presence of flavonoids and phenols (Table 1) suggests that the *C. spirale* has antioxidant, anti-allergic, anti-inflammatory, anti-microbial, anti-apoptosis, anti-ageing, anti-atherosclerosis, and anti-cancer activities. (Meenatchi and Jeyaprakash, 2015; Nielsen *et al.,* 2013). These phytochemicals are also known to possess biological properties such as cardiovascular protection and improvement of endothelial functions, as well as inhibition of angiogenesis and cell proliferation activities (Meenatchi and Jeyaprakash, 2015).

The high levels of tannins (polyphenols) in *C. spirale* would substantially contribute to the radical scavenging activity of *Croton spirale.* Polyphenols (such as tannins) are a major group of compounds that may act as primary antioxidants or free radical scavengers, hence block the action of free radicals, which have been implicated in the pathogenesis of many diseases and in the ageing process

(Meenatchi and Jeyaprakash, 2015). The radical scavenging capacities of these compounds have been used against heart diseases through reducing lipid oxidation. It was also hypothesized that the free radical scavenging properties of tannins may reduce the risk of cardiovascular diseases, cancer, blood clotting, and urinary tract infections (Bagchi *et al.,* 2000).

The presence of alkaloids and glycosides in the leaf extract also contribute to the antioxidant activity of *Croton spirale*, since plants extract containing alkaloids and glycosides are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements (Meenatchi and Jeyaprakash, 2015). These phytochemicals have been shown to exert a protective effect on oxidative neuronal damage through a scavenging action on reactive oxygen species (Zhang *et al.,* 2006: Maher and Davis, 1996; Kim *et al.,* 1999).

The presence of saponins also contribute to the antioxidant activity of *Croton spirale.* It has the potential to lower cholesterol levels in the human body due to their hypocholesterolemic effect (Nielsen *et al.,* 2013). They form complexes with cholesterol to reduce plasma cholesterol levels.

Phytic acid present in the leaf extract (Table 1) also contribute to the antioxidant activity of *Croton spirale.* This phytochemical has been recognized as a potent antioxidant because of its inhibitory effect on iron-catalyzed hydroxyl radical formation by chelating the iron required for generation of hydroxyl radical via the Fenton-type reaction, and iron-mediated oxidative damage involved in the progression of Parkinson's disease could be inhibited by phytates (Graf *et al.,* 1987; Obata, 2003; Dexter *et al.,* 1989). There is also evident that phytic acid is utilized as an antioxidant in food processing and storage to improve the oxidative stability of raw and cooked meat (Stodolak *et al.,* 2007). The levels of anti-nutrients in *Croton spirale* can be reduced by a number of processing methods like soaking, fermenting and boiling (Soetan and Oyewole, 2009), hence freeze-dried leaves decoction of *Croton* species are taken as tea by Filipinos (Gertrude, 2006).

The result for vitamin assay (Table 2) showed vitamins A and E, lipid soluble vitamins, to be present in appreciable quantities. They both conformed with the FAO/WHO standard. The high level of vitamin A seen in the result shows that *Croton spirale* is a good source of vitamin A. These vitamins have been shown to elicit good radical scavenging properties (Li, 2011; Panglossi, 2007). The B vitamins (B1, B2, B3, B6 and B12), water soluble antioxidants, were also present in considerable quantities, though higher than the FAO/WHO standard for these vitamins. Thus, their high values indicate that *Croton spirale* is a good source of B vitamins as they play important role in cell metabolism such as; slow down the progression of Alzeheimer's disease, co-enzymes of antioxidant enzymes, anti-inflammatory and depigmenting properties, inhibit lipid peroxidation, and many others (Hoey *et al.,* 2009; Bissette *et al.,* 2003; Keles *et al.,* 2010). The result for vitamin C was in conformity with the FAO/WHO standard for this vitamin.

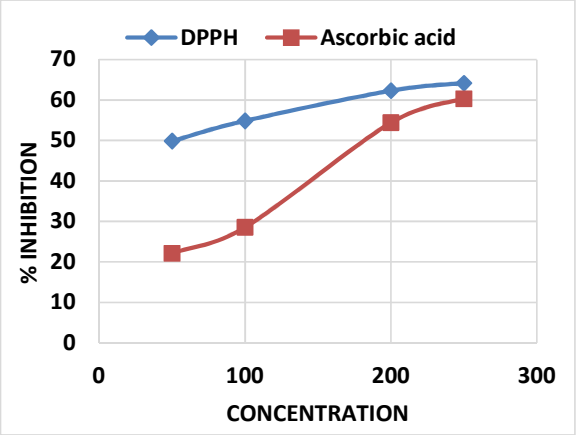

Fig. 1: Radical scavenging activity of *Croton spirale* leaf extract relative to standard ascorbic acid.

Table 1: Phytochemical profile of *Croton spirale*

Parameter	Concentration (%)
Flavonoids	13.16
Phenols	5.21
Tannins	128.32
Saponins	2.64
Alkaloids	11.92
Cardiac glycosides	2.00
Phytates	3.00
Oxalates	0.02

Table 2: Vitamin profile of *Croton spirale* compared with FAO/WHO standard for human vitamin requirements

Parameter	Concentration (mg/100)	FAO/WHO Standard for human vitamin requirements
Vitamin A	3.12	0.18 – 0.45
Vitamin B1	7.01	0.2 – 1.5
Vitamin B2	5.15	0.3 – 1.6
Vitamin B3	0.585	0.4 – 0.5
Vitamin B6	0.534	0.1 – 2.0
Vitamin B12	9.41	0.4 – 2.8
Vitamin C	6.231	5.0 – 70
Vitamin E	1.1503	0.2 – 2.7

Table 3: Mineral profile of *Croton spirale* compared with FAO/WHO standard for human mineral requirements

Parameter	Concentration (ppm)	FAO/WHO Standard for human mineral requirements
Zinc	1.906	0.5 – 3.0
Iron	3.366	0.58 – 3.27
Selenium	2.786	0.06 – 4.2

Furthermore, the result showed *Croton spirale* to contain appreciable amount of zinc, selenium and iron (Table 3), which conform to the FAO/WHO standard for these minerals. In living organisms, these minerals serve as enzyme cofactors for antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase. Antioxidant enzymes function by interfering with the oxidative processes by acting as electron donors (Cross *et al.,* 1987).

Further studies assessing the antioxidant activity of the extract by %inhibition of DPPH as compared to that of vitamin C was performed (Figure 1). It was found that the leaf extract has high DPPH scavenging activity, as compared to that of vitamin C. It was also found that the radical scavenging activity is dose-dependent, increasing

with increasing concentration. This appreciable scavenging ability recorded could be attributed to the presence of phenolic compounds, flavonoids, tannins,as well as the vitamins and minerals studied.

Conclusion

The results obtained in the present investigation revealed the presence of important phytochemicals, vitamins, and minerals present in methanolic extract of *Croton spirale*. The present study also demonstrated high DPPH scavenging activity relative to standard vitamin C. The findings of this study also suggest that *Croton spirale* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases.

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