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## Evaluation of bioactive constituents and *In vitro* antioxidant potentials of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata*

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### Abstract

The current study investigated the phytochemical constituents and *in vitro* antioxidant activities of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata*. The phytochemical analyses of the leaf extracts showed varying amounts of important compounds such as tannins, phenols, alkaloids, flavonoids, protein and carbohydrates. Findings from the *in-vitro* free radical mopping abilities of the ethanolic leaf extracts against 1,1-diphenyl-2-picryl hydrazyl and nitric oxide radical (NO<sup>•</sup>) as well as the reducing power and total antioxidant capacities revealed abilities to quell oxidative stress with *S. alata* > *E. hirta* > *D. mannii* excluding the NO<sup>•</sup> radical inhibition activity. This relationship was also reflected in the IC<sub>50</sub>/EC<sub>50</sub> values obtained, suggesting that the *S. alata* ethanolic leaf extract has the best antioxidant potential while *E. hirta* showed better activities than *D. mannii*. Thus, the leaves of *S. alata* could be further investigated for other biological potentials and possibly employed in the application of leaves against diseases that are implicated by oxidative stress.

**Keywords:** *Dracaena mannii*, *Euphorbia hirta*, *Senna alata*, phytochemicals, antioxidants, oxidative stress.

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## INTRODUCTION

Numerous bioactive compounds present in plants are synthesized from primary metabolites and have become the main source of herbal therapeutic products (Anigboro *et al.*, 2014; Al-Snafi, 2017). The medicinal properties of plants is dependent on the content of the phytochemical constituents (Essiet and Okoko, 2013) and have gained increased attention among rural dwellers as well as a great part of the civilized populations, for the maintenance of health (Anigboro, 2017; Anigboro *et al.*, 2018; Ghosh *et al.*, 2019; Anigboro *et al.*, 2019; Anigboro *et al.*, 2021). It is therefore important to determine the phytochemical constituents of such medicinal plants to further enhance their therapeutic efficacy and safety (Mohammed *et al.*, 2010).

*Dracaena mannii* (commonly called small-leaf dragon tree) is a small- to medium-size tree that is widespread in tropical Africa, and can record up to about 30 m and 2 m in height and diameter respectively (Damen *et al.*, 2018).

*Euphorbia hirta* is a small pan tropical erect annual herb with hairy stem that grows up to 50cm, and is commonly found in North East Coast of Tamil Nadu and South Western Ghats of India as well as Nigeria (Essiet and Okoko, 2013). *Senna alata* (commonly called candle bush or Christmas candles) is both an ornamental and medicinal plant that is natively found in the tropics (Adedayo *et al.*, 2001). It is locally called “Nelkhi” and “Asunwo oyibo” in eastern and western Nigeria respectively (Mutiu *et al.*, 2015). While flowers of *Senna alata* and leaf of *Euphorbia hirta* had been reported to possess antimicrobial, antifungal, anti-inflammatory, laxative and analgesic effects (Adedayo *et al.*, 2001; Adjero *et al.*, 2015; Ilodibia *et al.*, 2015; Al-Snafi, 2017; Damen *et al.*, 2018; Thu *et al.*, 2020), there is still limited information on the relative antioxidant capacities of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* (Figure 1). Hence, the present investigation explored the comparative phytochemical constituents and *in vitro* capacity of the ethanolic leaf extracts of *D. mannii*, *E. hirta* and *S. alata*.



Figure 1: The Plants' Leaves: A = *Dracaena mannii*; B = *Euphorbia hirta*; C = *Senna alata*.

## MATERIALS AND METHODS

### Chemicals/reagents used

All the chemicals and reagents that were used in the various biochemical processes are of analytical quality.

### Collection and authentication of plant materials

Fresh leaves of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* were collected from bushes at Ovirie-Ovu, Okpe-Olomu and Abraka in Delta State, Nigeria. Before use, the

plants' specimens were identified at the Department of Plant Biology and Biotechnology (with the Voucher number; UBH-D019, UBH-E023 and UBH-S491 respectively), University of Benin, Edo State, Nigeria.

### Preparation of plant extract

The modified method of Anigboro *et al.* (2019) was used for the preparation of the plant's leaf extracts. Summarily, the fresh leaves of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* were rinsed with tap water to remove dirt, air-dried under shade for about two weeks and ground to coarse powder using an electric

blender, from which 100g of coarsely powdered leaves were soaked in 800 mL of ethanol for 48 hours (with stirring at 24 hours intervals). The extract solutions were filtered firstly with a double-layer cheese cloth and thereafter with filter paper (Whatman No. 1). The filtrates obtained were concentrated in a rotary evaporator at 50°C for two hours and then evaporated to dryness over water bath (50°C). The yield percent of the extraction process were evaluated using equation 1 and the obtained dried extracts were stored at 4°C in air-tight sterile containers till when needed for analysis.

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract (W1)} \times 100}{\text{Weight of ground leave (W2)}}$$

### Qualitative phytochemical screening

The standard protocols reported by Njoku and Obi (2009) and Borokini and Omotayo (2012) were used for the preliminary phytochemical screening of the various leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata*, for the presence of phlobatannins, cardiac glycosides, flavonoids, saponins, terpenes/terpenoids, carbohydrates, tannins, phenols, steroids, proteins and alkaloids.

### Quantitative phytochemical analysis

#### Estimation of phenols and tannin contents

The method of Singleton and Rossi (1965) was employed for the evaluation of the phenol and tannin contents of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata*. To a mixture of the extracts' solutions (1.0 mL; 0.02 g/mL) and Folin-Ciocalteu's reagent (1.0 mL; 1:10 v/v with water) which was allowed to stand for three minutes, a saturated solution of Na<sub>2</sub>CO<sub>3</sub> (1.0 mL) and distilled water (10.0 mL) were added and kept in the dark for 90 minutes after which the optical densities (OD) were read with the Thermo-Fisher G10S (TFG) UV – Vis Spectrophotometer at 725 nm. Different concentrations (20 – 100 µg/mL) of gallic acid and tannic acid were used to generate calibration curves from which the amounts of phenols and tannins were respectively deduced from the graphs [expressed as gallic acid equivalent (GAE) tannic acid (TAE) equivalents/g extract] respectively.

#### Estimation of total flavonoids

The standard method of Jia *et al.* (1999) was used for the estimation of the flavonoid contents of the extracts. A mixture of the various extract solutions (0.5 mL; 0.02 g/mL), NaNO<sub>2</sub> (0.075 mL; 5 %) and distilled water (1.25 mL) was incubated for 5 minutes at room temperature. Thereafter, AlCl<sub>3</sub> (0.15 mL; 10 %) was added and incubated again for 6 minutes followed by addition of NaOH (0.5 mL; 1.0 M) and dilution with distilled water (0.275 mL). The OD were read immediately at 510 nm using the TFG UV–Vis Spectrophotometer. Catechin was used at different concentrations (20 – 100 µg/mL) to generate a standard calibration curve, from which the flavonoids content was deduced and expressed as equivalents of catechin (mg/g) .

### Determination of carbohydrates contents

The Miller method (1959) was used for the estimation of carbohydrates contents of the various extracts. The extracts' solutions (1.5 mL; 0.02 g/mL) were mixed DNSA reagent (1.5 mL) and incubated at 90°C over water for 10 minutes. Potassium sodium tartrate solution (0.5 mL; 40%) was added and cooled to room temperature before reading the OD at 575 nm using the TFG UV–Vis Spectrophotometer. Using a standard calibration graph of glucose concentrations (0.2 – 1.0 mg/mL), the total carbohydrates content was presented as glucose equivalent/g extracts.

### Total protein determination

Protein contents of sample extracts was assessed by employing the Biuret method (Gornall *et al.*, 1949). A mixture of the extracts' samples (1.0 mL; 0.02 g/mL) and Biuret reagent (3.0 mL) was incubated for 30 minutes at room temperature, and read at 540 nm using the TFG UV–Vis Spectrophotometer. Bovine serum albumin (BSA) (200 – 1000 µg/mL) was used to obtain a standard calibration graph, from which the protein contents of the samples were deduced.

### Estimation of alkaloids contents

The alkaloids contents of extract samples was evaluated using the standard procedure reported by Shamsa *et al.* (2008). A mixture of the extracts' samples (0.5 mL), phosphate buffer (2.5 mL; pH 4.7; 2.0 M), bromocresol green solution (2.5 mL) and chloroform (4.5 mL) was read spectrophotometrically at 470 nm against blank. Atropine (40 – 120 µg/mL) was used as a standard compound to obtain a standard calibration graph, from which the

alkaloid contents were presented as atropine equivalents.

### Determination of phytate contents

The standard procedure reported by Wilcox *et al.* (2000) was used for the estimation of the phytate contents of the extracts. The extracts' samples (1.0 mL) were vortexed thoroughly with HCl (1.0 mL; 0.4mM) followed by centrifugation at 3000rpm for 5 minutes. To the supernatants (0.1 mL), distilled water (0.9 mL) and 1.0 mL of the colour reagent (3.0 M sulfuric acid, 2.5%w/v ammonium molybdate and 10% ascorbic acid mixed in equal volume with two equal volume of distilled water) were added, followed by incubation for one hour at room temperature. The optical densities were measured against blank at 650 nm with TFG UV-Vis Spectrophotometer. Potassium dihydrogen phosphate was used as standard compound to obtained a standard calibration graph, from which the alkaloid contents were presented as its equivalent.

### *In vitro* free radical quelling and antioxidants activities

#### 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) Radical Inhibitory Assay

The free radical inhibitory abilities of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* were assessed using the standard procedure reported by Manzocco *et al.* (1998). A mixture of the extracts' solutions (1.0 mL; 1.0 – 7.0 mg/mL) and DPPH solution (2.0 mL; 0.3 mM) was incubated in the dark for 30 minutes and read spectrophotometrically at 517 nm. The inhibition of DPPH radical was calculated in percentage using the equation (2) below:

$$\text{Radical Inhibition (\%)} = \frac{[A_c - A_s]}{A_c} \times 100$$

..... Equation 2.

Where  $A_s$  and  $A_c$  are the respective absorbance values of the sample extract and the control (which was carried out as described above but using distilled water in lieu of the extract sample). The effective concentration that results to 50% inhibition of DPPH radical ( $IC_{50}$ ) was deduced graphically.

#### Nitric oxide radical ( $NO^*$ ) inhibitory assay

The  $NO^*$  inhibitory potentials of the extracts' samples was measured using the standard procedure reported by Marcocci *et al.* (1994). A

mixture of the extracts' solutions (1.0 mL; 1.0 – 7.0 mg/mL), sodium nitroprusside (2.0 mL; 10.0 mM) and phosphate buffer saline (0.5 mL; 10 mM; pH 7.4) was incubated for 150 minutes at 25°C, from which 0.50 mL was mixed with 1.0 mL of 0.33% sulfanilic acid in 20% glacial acetic acid, followed incubation at 25°C for 5 minutes. Naphthylethylenediamine dichloride (1.0 mL; 0.1 %w/v) was added before another incubation at 25°C for 30 minutes. The absorbance were then measured at 546 nm against blank using the Thermo-Fisher G10S UV-Vis Spectrophotometer and the percentage inhibition of nitric oxide radical was calculated using the equation (2). The effective concentration that resulted to 50% inhibition of  $NO^*$  ( $IC_{50}$ ) was deduced graphically.

#### Reducing power (rp) assay

The RP of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* was assessed using the method of Oyaizu (1986). Phosphate buffer (2.5 mL; 0.2 M; pH 6.6) and  $K_3Fe(CN)_6$  (2.5 mL; 1.0 %) were added to the extracts' samples (1.0 mL; 1.0 – 7.0 mg/mL), and incubated at 50 °C for 20 minutes, thereafter Trichloroacetic acid (2.5 mL; 10% w/v) was added. The mixtures were centrifuged at 3000 rpm for 10 minutes, and the supernatants (2.5 mL), mixed with distilled water (2.5 mL) and of  $FeCl_3$  (0.5 mL; 0.10%), was read spectrophotometrically at 700 nm against blank . The effective concentration that results to RP absorbance of 0.5 ( $EC_{50}$ ) was deduced graphically.

#### Assessment of total antioxidant capacity

The TAC of the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* were assessed using the method Prieto *et al.* (1999).A mixture of the extracts' samples (0.1 mL; 1.0 – 7.0 mg/mL) and 1.0 mL of the working reagent (mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was incubated at 95°C for 90 minutes. After cooling to room temperature, the OD was measured at 695 nm against blank using the TFG UV-Vis Spectrophotometer. Gallic acid (GA) (20 – 100 µg/mL) was used as standard antioxidant compound to generate a calibration graph from which were the TAC was deduced as GA equivalents.

#### Statistical analysis

All data obtained from the various biochemical procedures were analysed by One-way ANOVA using the SPSS-PC programme software while graphical works were done with Microsoft Excel. The values were presented as Mean  $\pm$  Standard deviation while significance was considered at p-values of less than 0.05.

## RESULTS AND DISCUSSION

Plants are known to be treatment source in both ethno-therapeutic and conventional medicine and are mostly employed as alternative therapy since they contain phytochemicals which are secondary metabolites in one or more parts of the plants and also due to several advantages ranging from cost, minimal side effects to high efficacy (Anigboro, 2018; Anigboro *et al.*, 2018; Anigboro *et al.*, 2019; Anigboro *et al.*, 2020). The phytochemicals present in plants play certain physiological roles in plants and have the ability to produce definite physiological action in human body (Dzialo *et al.*, 2016; Mahmoudi *et al.*, 2016; Nyamai *et al.*, 2016; Anigboro *et al.*, 2020).

In this study, the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata* were shown to possess phytochemicals at varying degrees both qualitatively and quantitatively. From the result obtained in this study (Table 1), saponin was found only in the *D. mannii* and *S. alata* extracts; cardiac glycosides were only found in the *E. hirta* and *S. alata* extracts; terpenes, steroids and phlobatannins were absent in all three extracts; while tannins, alkaloids, flavonoids, phenols, carbohydrates and proteins were present in all three extracts. Also, there was variation in the quantities of the commonly present bioactive compounds in these extracts (Table 2) such that: phenol and flavonoids contents in *S. alata* extract are significantly higher than in the *E. hirta* extract which in turn has higher amounts than the *D. mannii* extract ( $p < 0.05$ ). The order of tannins, alkaloids and phytate contents is such that: *E. hirta* > *S. alata* > *D. mannii*. These phytochemical compounds have previously been reported to possess pharmacological properties.

**Table 1:** Yield and Qualitative Phytochemical Constituents of Ethanolic Leaf Extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata*.

	<i>Dracaena mannii</i>	<i>Euphorbita hirta</i>	<i>Senna alata</i>
Yield (%)	10.93	4.44	11.70
Compounds			
Saponin	+	-	+
Tannin	+	+	+
Terperne	-	-	-
Steroid	-	-	-
Alkaloid	+	+	+
Phlobatanin	-	-	-
Cardiac glycoside	-	+	+
Flavonoid	+	+	+
Phenol	+	+	+
Carbohydrate	+	+	+
Protein	+	+	+

Key: + = Present; - = Absent.

Antioxidant activity is essential in terminating free radicals generation as well as diseases associated with same (Sharma *et al.*, 2008). The DPPH radical inhibition assays as well as RP and TAC assays in this study revealed the ethanolic leaf extracts' of *S. alata* > *E. hirta* > *D. manni* and may have the ability to quell oxidative stress while the NO<sup>\*</sup> inhibition assay had a different pattern. This relationship was also reflected in the IC<sub>50</sub>/EC<sub>50</sub> values that were deduced from the graphs thus, suggesting the

*S. alata* ethanolic leaf extract as possessing the best antioxidant potential while the *E. hirta* is better than *D. mannii* (Figures 2,3,4 and 5). This antioxidative order may be attributed to the amounts of phenolic and flavonoid compounds in the leaf extracts (Table 2). Phenolic and flavonoids compounds are reportedly known to exert antioxidant property (Mohammed *et al.*, 2010; Dzialo *et al.*, 2016; Mahmoudi *et al.*, 2016; Thu *et al.*, 2020).

Table 2: Quantitative Phytochemical Constituents of Ethanolic Leaf Extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata*.

Compounds	<i>Dracaena mannii</i>	<i>Euphorbita hirta</i>	<i>Senna alata</i>
Phenols (mg/gGAE)	11.90± 0.98 <sup>a</sup>	91.01± 1.58 <sup>b</sup>	80.60± 1.04 <sup>c</sup>
Flavonoid (mg/gCAE)	7.82 ± 0.03 <sup>a</sup>	37.01± 0.65 <sup>b</sup>	62.70± 3.56 <sup>c</sup>
Tannin (mg/gTAE)	22.81± 0.59 <sup>a</sup>	107.02± 1.18 <sup>b</sup>	95.11± 1.23 <sup>c</sup>
Alkaloid (mg/gATE)	4.18 ± 1.16 <sup>a</sup>	17.99 ± 0.48 <sup>b</sup>	16.72± 0.11 <sup>c</sup>
Carbohydrates (mg/gGluE)	288.03± 0.17 <sup>a</sup>	107.04± 3.12 <sup>b</sup>	205.01± 7.82 <sup>c</sup>
Protein (mg/g)	0.24 ± 0.02 <sup>a</sup>	1.59 ± 0.06 <sup>b</sup>	6.23 ± 0.19 <sup>c</sup>
Phytate (mg/g)	0.15 ± 0.04 <sup>a</sup>	1.28 ± 0.26 <sup>b</sup>	1.06 ± 0.18 <sup>c</sup>

Values are presented as means ± standard deviations (n = 3). Values with distinct superscript alphabets on the same row are significantly different (p<0.05). Key: GAE = Gallic acid equivalent, CAE = Catechin equivalent, TAE = Tannic acid equivalent, ATE = Atropine equivalent, GluE = Glucose equivalent.

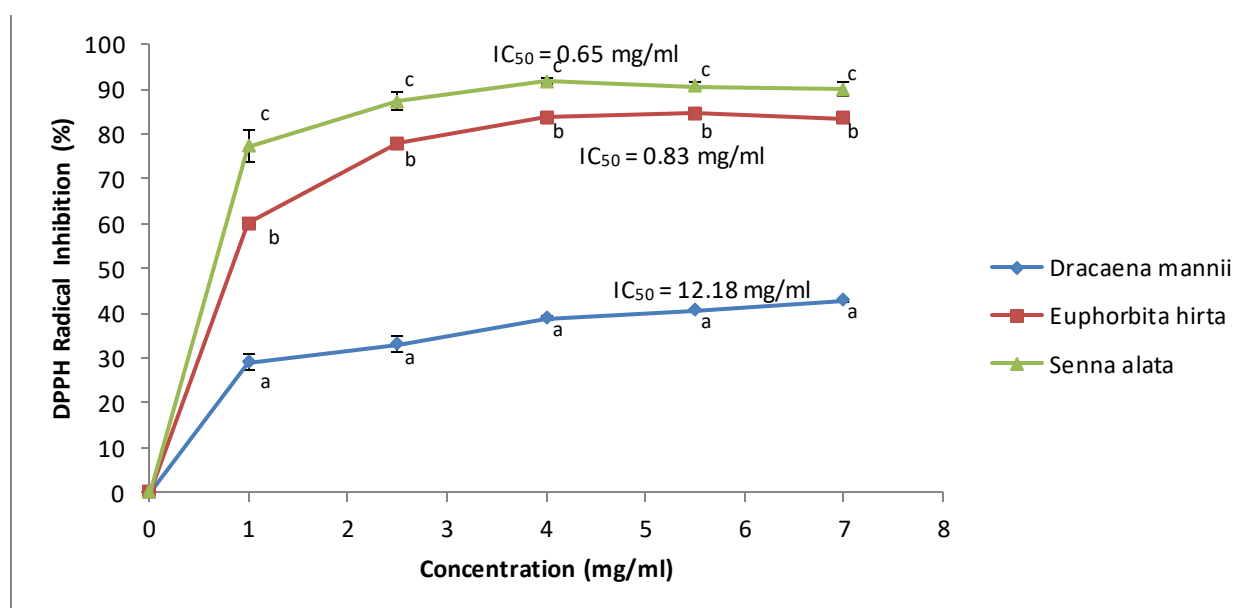


Figure 2: Inhibition of DPPH Radical by Ethanolic Leaf Extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata*. Values are means ± standard deviations (n = 3). Data points (along a common concentration value) with different attached letters are significantly different (p<0.05).

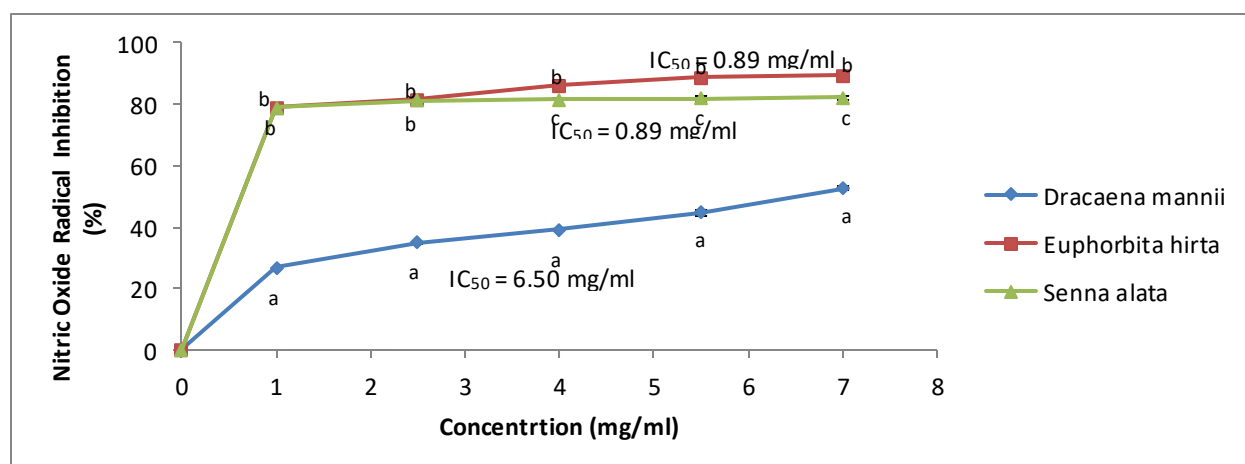


Figure 3: Inhibition of NO<sup>•</sup> by Ethanolic Leaf Extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata*. Values are means ± standard deviations (n = 3). Data points (along a common concentration value) with different attached letters are significantly different (p<0.05).

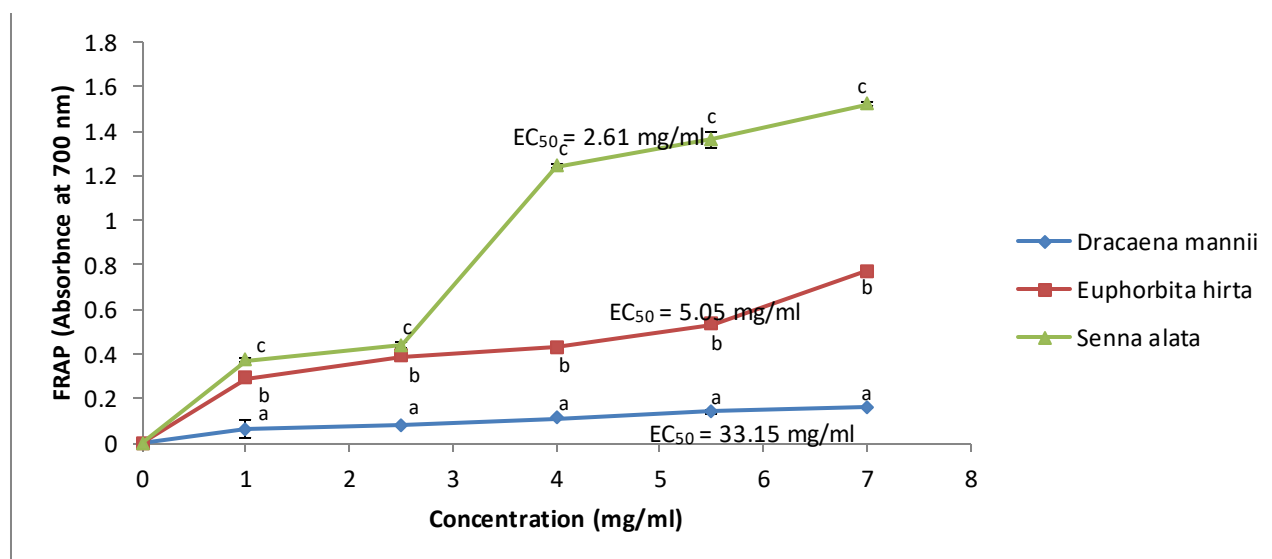


Figure 4: Reducing Power of Ethanolic Leaf Extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata*. Values are means ± standard deviations (n = 3). Data points (along a common concentration value) with different attached letters are significantly different (p<0.05).

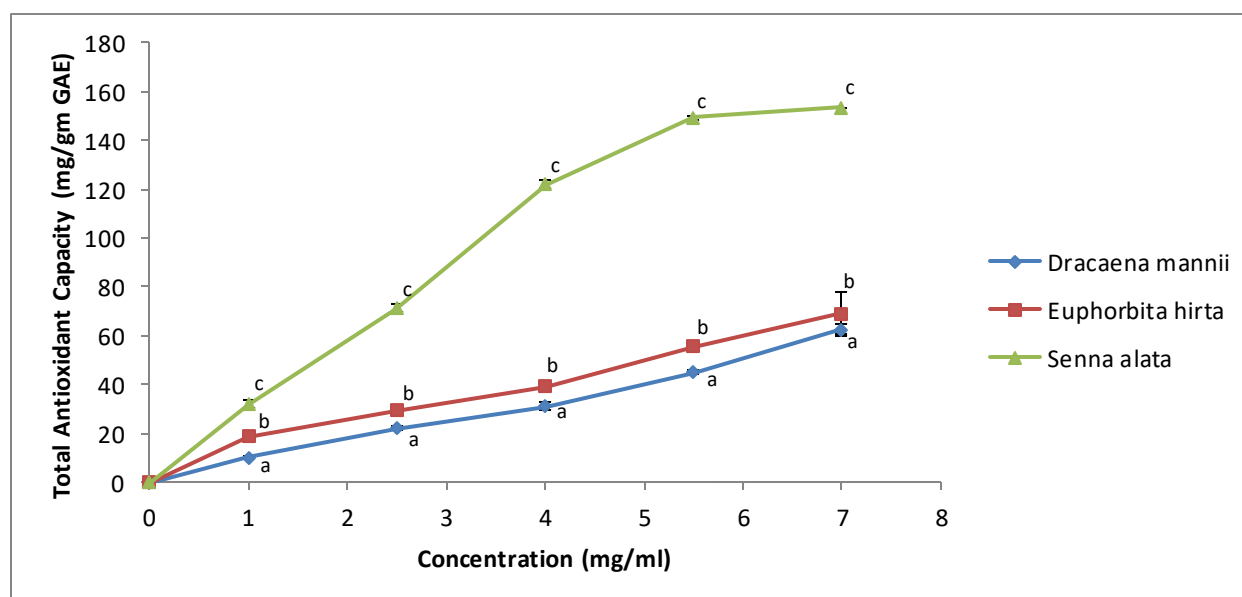


Figure 5: Total Antioxidant Capacity of Ethanolic Leaf Extracts of *Dracaena mannii*, *Euphorbita hirta* and *Senna alata*. Values are means ± standard deviations (n = 3). Data points (along a common concentration value) with different attached letters are significantly different (p<0.05).

Several medicinal plant extracts which have been researched and reported in literature to exhibit biological activities against diabetes

mellitus, obesity, inflammation, malaria, bacterial and fungal infections, etc are also known to be quite rich in phytochemicals and

possess high antioxidant capacities (Mohammad *et al.*, 2010; Dzialo *et al.*, 2016; Anigboro *et al.*, 2018, 2020,2021). This suggests the likely medicinal benefits of the ethanolic leaf extracts of *S. alata*, *E. hirta*, and *D. Mannii* reported in this study, especially against disease conditions in which oxidative stress is implicated.

## CONCLUSION

In the present study, the ethanolic leaf extracts of *Dracaena mannii*, *Euphorbia hirta* and *Senna alata* were shown to possess phytochemicals at varying quantities. However, *S. alata* expressed a higher *in vitro* antioxidant capacity as compared with *E. hirta* and *D. mannii*. Thus, the leaves of *S. alata* could be further investigated for its antioxidant potentials in addition to other biological potentials and be possibly employed in the application leaves against diseases that are implicated by oxidative stress.

## Conflict of interest

The authors declare that they have no known competing interests that could appear to influence the work reported in this paper.

## Author contribution

The experiment was conceived and designed by AAA, OJA and NJT. OA carried out the experimental work; OA, AAA and OJA analyzed the data; OA, AAA, OJA and NJT contributed reagents, materials and provided the resources used for analysis; OJA, OA and AAA undertook the manuscript writing and proofreading; while AAA, OJA and NJT supervised the experiments and revised the manuscript. All authors approved the final draft of the manuscript.

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