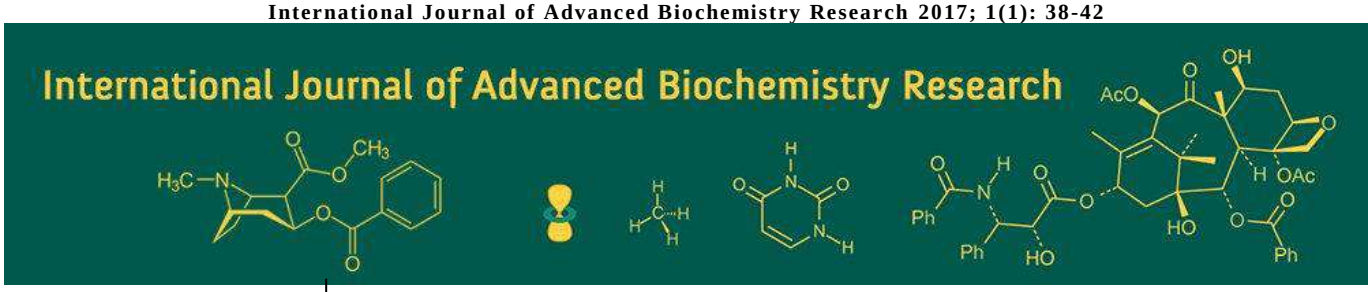

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Effect of *Anthocleista vogelii* leaves on free radicals: Antioxidant studies

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Abstract
In traditional medicine, numerous diseases are treated using the leaves of *Anthocleista vogelii*. The total phenolic content and flavonoid content (antioxidants); and the effect of leaves extracts (ethanolic extract and fractions [*n*-hexane, dichloromethane and ethyl acetate]) of *Anthocleista vogelii* were determined in this study. The free radical scavenging property of the extracts were investigated using nitric oxide, hydrogen peroxide and 1, 1-Diphenyl-2-picrylhydrazyl assays. Spectrophotometric method was used for evaluating the antioxidant property of *Anthocleista vogelii* leaves. In this study, the results showed that *Anthocleista vogelii* leaves extracts exerted antioxidant and free radical scavenging activities. Total Phenol and flavonoid were present in the leaves extract. Ethyl acetate fraction exerted a more free radical scavenging effect more than other extracts of *Anthocleista vogelii* leaves. Ehtyl acetate fraction scavenged nitric oxide (68.19%), hydrogen peroxide (80.0%) and 1, 1-Diphenyl-2-picrylhydrazyl (92.02%). Ethyl acetate fraction also had the highest total antioxidant capacity (70.69 mg AAE/g), phenol (55.17 mg GAE/g) and flavonoid (60.23 mg QE/g) content than other extracts of *Anthocleista vogelii* leaves. The results from this study conclude that, *Anthocleista vogelii* leaves extracts (ethanolic extract and fractions [*n*-hexane, dichloromethane and ethyl acetate]) have antioxidant activities and ethyl acetate fraction exerted more antioxidant activity.

Keywords: *Anthocleista vogelii*, leaves, free radicals, antioxidants, phenols

Introduction
Free radicals such as reactive nitrogen and oxygen species are produced during oxidative reactions in the body and they help in normal processes going on in body cells [1]. Free radicals at higher levels can damage cellular components and these may cause diseases such as stroke, cancer, atherosclerosis, aging and diabetes [2-5]. Diseases caused by free radicals may be treated with medicinal plants that have antioxidant and free radicals scavenging properties [6, 7]. Antioxidants help in neutralizing free radicals (nitrogen and oxygen species) which are responsible for causing diseases such as cancer, arthritis and diabetes [8]. Recently, there is an increase in research on therapeutic effect of medicinal plants with antioxidant due to their free radical scavenging effect [9, 10].
Anthocleista vogelii belonging to the family Loganiaceae is a plant used in ethno-medicine for the treatment of various diseases which includes fever, syphilis, pile and stomach ache [11, 12]. Previous studies reported that *Anthocleista vogelii* root exerted antidiabetic effect in albino rats that were induced with diabetes using streptozocin and alloxan [13, 14]. Previous studies also reported the presence of phenolic content and antioxidant property of *Anthocleista vogelii* root [11]. The antioxidant and free radical scavenging activities of *Anthocleista vogelii* leaves extracts (ethanolic extracts and fractions) were determined in this study.

Materials and Methods
Anthocleista vogelii leaves were gotten from Bioresources Development Centre (BIODEC), Ogbomoso, Nigeria. The plant was identified at the herbarium, Botany Department, Obafemi Awolowo University, Ile-Ife Nigeria and a sample of the plant leaf with voucher number; IFE 17399 was deposited at the herbarium.

Extraction
Anthocleista vogelii leaves were washed under clean running tap water, and thereafter dried

in the oven for 7 days at 40 °C. A grinder was used to pulverize the dried plant sample before soaking the sample in 70% ethanol. After 72 hours, it was filtered using Whatman no. 1 filter paper and then a rotary evaporator was used to concentrate the sample into a solid paste. Thereafter, the solid paste was dried using a freeze dryer in order to obtain a dried ethanolic extract [15]. The dried ethanolic extract of the leaves of *Anthocleista vogelii* was fractionated using *n*-hexane, followed by dichloromethane and then ethyl acetate [15, 16]. The fractions each were concentrated, dried, labeled and stored at 4 °C in a refrigerator before proceeding with the study.

Free Radical Scavenging Assays

1,1-diphenyl-2-picrylhydrazyl hydrate assay

1ml of 0.3 mM 1,1-diphenyl-2-picrylhydrazyl hydrate dissolved in methanol was added to increasing concentration of the plant samples (extract and fractions) and standard (ascorbic acid) each dissolved in methanol. It was then incubated for 30 minutes in the dark at room temperature and the absorbance was read at 517 nm [17,18]. The radical scavenging activity (%) was determined using Equation (i):

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance}_{RB} - (\text{Absorbance}_{S} - \text{Absorbance}_{SB})}{\text{Absorbance}_{RB}} \text{ X } 100$$

Where

RB = Reagent Blank, S = Sample and SB = Sample Blank

Nitric oxide assay

0.5 ml of Sodium nitroprusside (10 mM) dissolved in phosphate buffered saline (0.2 M and pH 7.4) was poured in 1.0 ml of increasing concentration of the plant samples each dissolved in methanol. The mixture was incubated at 25 ºC for 3 hours and then Griess reagent (sulfanilamide [1%], naphthyl ethylenediamine dihydrochloride [0.1%] in phosphoric acid [2.5%]) at 1.5 ml was added. The absorbance was read at 546 nm [19, 20]. The radical scavenging activity (%) was determined using Equation (i).

Hydrogen peroxide assay

0.6 ml of 43 mM hydrogen peroxide prepared in phosphate buffer (1.0 M and pH 7.4) was added to increasing concentration of the plant samples each dissolved in methanol. After 10 minutes, absorbance was measured at 230 nm after 10 min [7]. The radical scavenging activity (%) was determined using Equation (i).

Total antioxidant capacity assay

1.0 ml of the samples each were added to 1.0 ml of reagent solution (sulphuric acid [0.6 M], sodium phosphate [28 mM] and ammonium molybdate [4 mM]) in different test tubes and then incubated for 1 hour, 30 minutes at 95 ^{O}C in a water bath. The absorbance was read at 695 nm after cooling the mixture [7]. Total antioxidant capacity of the samples in mg standard (ascorbic acid) equivalent g^{-1} (mg AAE/g) of sample was determined using Equation (ii):

$$\text{TAC} = \frac{\text{C} \cdot \text{V}}{\text{M}}$$

Where: TAC = Total antioxidant in mg standard, C = Concentration of standard established from calibration curve (mg/ml), V = Volume of the extract (ml) and M = Weight of the extract (g)

Total phenolic content assay

0.2 ml of Folin's reagent was added to a mixture made up of 0.1 ml of sample (1 mg/ml) and 0.9 ml distilled water. Thereafter it was vortexed and after 5 minutes, 1.0 ml of 7% (w/v) Na_2CO_3 solution was added and then incubated for 90 minutes at room temperature. Absorbance was measured at 750 nm [21]. The total phenolic content of the extracts expressed as mg standard (gallic acid) equivalent g^{-1} (mg GAE/g) of extract was calculated using Equation (ii).

Total flavonoid content assay

0.4 ml of distilled water was added to 0.1 ml of sample (1 mg/ml) and then 0.1 ml of sodium nitrite (5%). 5 minutes later, 0.1 ml of aluminum chloride (10%) and 0.2 ml sodium hydroxide (1 M) was added to the mixture. Absorbance was read at 510 nm [21]. The total flavonoid contents expressed as mg standard (Quercetin) equivalents g^{-1} (mg QE/g) of plant extract was calculated using Equation (ii).

Statistical Analysis

One-way analysis of variance followed by Bonferroni t-test post hoc comparisons was used to determine the significant difference at 95% ($p< 0.05$) using version 3.01 of Primer. Graph plotted on excel was used in determining 50% inhibition concentration (IC_{50}) of the samples.

Results

***Anthocleista vogelii* leaves radical scavenging effect**

Anthocleista vogelii leaf extracts at 500 µg exerted free radical scavenging activity (Table 1). 100 µg of the standard drug (ascorbic acid) significantly ($P< 0.05$) scavenged free radicals higher than *A. vogelii* leaf extracts (Table 1). Lower half maximal inhibitory concentration (IC_{50}) of the ethyl acetate fraction significantly ($P< 0.05$) caused a higher significant ($P< 0.05$) radicals scavenging effect than other extracts of *A. vogelii* leaf (Table 1 and Table 2).

Table 1: Effect of *Anthocleista vogelii* leaves extracts on free radicals

Samples	1,1-diphenyl-2-picrylhydrazyl hydrate (%)	Nitric oxide (%)	Hydrogen peroxide (%)
500 µg AVE	80.77 ± 0.13 *#	70.90 ± 0.10 *#	59.67 ± 0.11 *#
500 µg Dichloromethane fraction	70.82 ± 0.12 *#	62.10 ± 0.06 *#	50.04 ± 0.10 *#
500 µg Ethyl acetate fraction	92.02 ± 0.14 #	80.00 ± 0.08 #	68.19 ± 0.09#
500 µg *n*-Hexane fraction	61.17 ± 0.10*#	54.22 ± 0.07 *#	41.07 ± 0.10 *#
100 µg Ascorbic acid	98.32 ± 0.11	92.11± 0.09	93.44 ± 0.12

Values are mean ± SEM; n = 3. AVE: *Anthocleista vogelii* leaves Ethanolic Extract; DPPH: 1,1-diphenyl-2-picrylhydrazyl hydrate. * Significant at $P< 0.05$ when compared to Ethyl acetate fraction. # Significant at $P< 0.05$ when compared to ascorbic acid

Table 2: Half maximal inhibitory concentration (IC_{50}) of *Anthocleista vogelii* leaves extracts

***Anthocleista vogelii* leaves extracts**	**1,1-diphenyl-2-picrylhydrazyl hydrate (IC_{50})**	**Nitric oxide (IC_{50})**	**Hydrogen peroxide (IC_{50})**
Ethanolic extract (µg/ml)	238.12 ± 0.10 *	298.22 ± 0.24 *	389.58 ± 0.17 *
Dichloromethane fraction (µg/ml)	224.07 ± 0.08 *	274.30 ± 0.20 *	372.98 ± 0.11 *
Ethyl acetate fraction (µg/ml)	210.00 ± 0.04	217.82 ± 0.21	361.08 ± 0.14
n-Hexane fraction (µg/ml)	254.78 ± 0.06 *	328.12 ± 0.23 *	412.90 ± 0.16 *

Values are mean ± SEM; n = 3; IC_{50}: half maximal inhibitory concentration. * Significant at $P< 0.05$ when compared to Ethyl acetate fraction.

***Anthocleista vogelii* leaves extracts antioxidant property**

Anthocleista vogelii leaves extracts exerted potent antioxidant properties including total flavonoids and phenol content. The ethyl acetate extract fraction exerted a higher (significant at $p< 0.05$) antioxidant activity when compared to other extracts of *A. vogelii* leaves (Fig. 1).

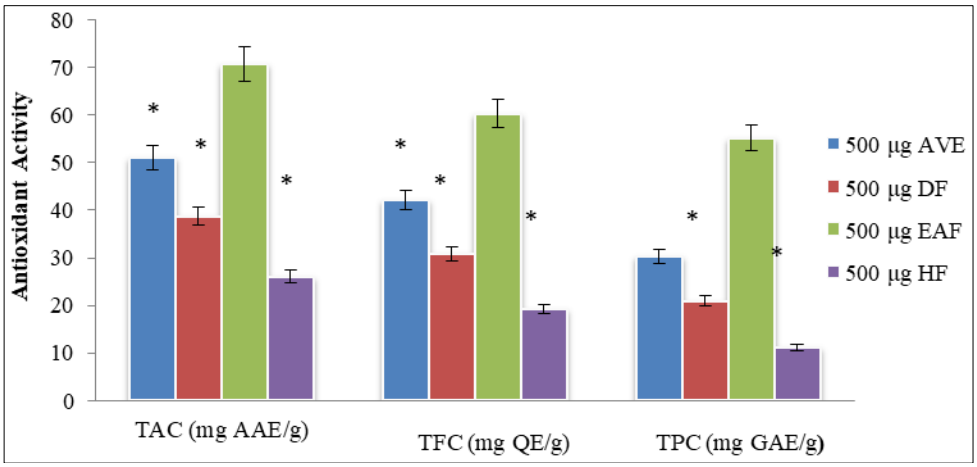

Fig 1: Antioxidant property of *Anthocleista vogelii* leaves ethanolic extracts

Data are represented as mean ± SEM; n = 3. AVE: *Anthocleista vogelii* leaves ethanolic extract; DF is dichloromethane extract fraction; EAF is Ethyl acetate extract fraction; HF is *n*-hexane extract fraction. TAC is Total antioxidant content; TFC is Total flavonoid content and TPC: Total phenol content. * Significant at $P< 0.05$ when compared to Ethyl acetate fraction.

Discussion

The leaves of *Anthocleista vogelii* leaves extracts (ethanolic extract and extract fractions [ethyl acetate fraction, dichloromethane fraction and *n*-hexane fraction]) scavenged free radicals (Table 1). The antioxidants; flavonoid and phenol were also present in *A. vogelii* leaves extracts as shown in Fig. 1. Lower half maximal inhibitory concentration (IC_{50}) of ethyl acetate fraction of *A. vogelii* leaves ethanolic extract scavenged free radicals more than other extracts of *A. vogelii* leaves (Table 1 and Table 2). The phenol and flavonoid content of the ethyl acetate fraction of *A. vogelii* leaves ethanolic extract was also more than other extracts of *A. vogelii* leaves (Fig. 1). The results of these study is similar to earlier study that reported that the root of *Anthocleista vogelii* extracts (ethanolic extract and extract fractions [aqueous fraction, ethyl acetate fraction, dichloromethane fraction and *n*-hexane fraction]) scavenged free radicals and it had phenol and flavonoid [22]. Another study reported that *Anthocleista vogelii* leaves methanolic extract exerted antioxidant and free radical-scavenging activities [23]. Numerous research works have been done over the years to determine the biological activities of different solvent extracts of *Anthocleista vogelii.* These previous studies reported that *Anthocleista vogelii* exerted analgesic, antidiabetic [13, 14], anti-plasmodial [24], antibacterial [25] and anti-fungal [26] effects. Earlier research works reported that some plants exert therapeutic properties which include analgesic [27], anti-inflammatory [28], anticancer [29, 30] and antidiabetic [15, 31] effect due to their antixodant activities.

This study showed that *Anthocleista vogelii* leaves extracts (ethanolic extract and extract fractions [ethyl acetate fraction, dichloromethane fraction and *n*-hexane fraction]) scavenged free radicals; and antioxidants (phenol and flavonoid) were present (Table 1, Table 2 and Fig. 1). Thus, further studies on *Anthocleista vogelii* leaves may lead to the discovery of new medicines that can be used for the treatment of diseases caused by free radicals.

Conclusion

The results shows that *Anthocleista vogelii* leaves extracts (ethanolic extract and extract fractions [ethyl acetate fraction, dichloromethane fraction and *n*-hexane fraction]) have antioxidant properties which include free radicals scavenging effect.

Conflicts of interests: No conflicts of interests exist.

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