



Evaluation of antioxidant and cytotoxic activities of *Cleome gynandra*

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Abstract

Background: *Cleome gynandra* (Cleomaceae) is a plant species distributed throughout most parts of tropical regions of the world and has a number of medicinal uses; the plant is used to treat typhoid, diarrhea, ear pain and anemia. **Objective:** To investigate the cytotoxicity and antioxidant potentials of ethanol, *n*-hexane, chloroform and 60% aqueous methanol extracts of *Cleome gynandra*. **Method:** Air dried powdered plant material (500 g) was defatted using *n*-hexane (2 L), the residue was percolated with ethanol (2 L) for 10 days. The ethanol extract was partitioned using *n*-hexane, chloroform and 60% aqueous methanol gradient. In this study, the antioxidant activities of the extracts of *C. gynandra* were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate techniques. The cytotoxic activities of the plant were investigated using *Artemia salina* (brine shrimp) bioassay. **Results:** The extracts showed significant antioxidant activity against DPPH radical with IC₅₀ values of 137.5, 370.3 µg/mL for aqueous methanol, and ethanol fraction respectively while the IC₅₀ of *n*-hexane and chloroform fraction is >1000 µg/mL. All the test samples have shown strong activity in the brine shrimp assay with LC₅₀ values of 7.19, 14.80, 72.09 and 76.09 µg/mL for *n*-hexane, ethanol chloroform and aqueous methanol fraction respectively. **Conclusion:** All the tested extracts showed significant antioxidant and cytotoxic properties, suggesting anti-inflammatory and anti-tumor potency of the plant.

Keywords: *Cleome gynandra*, cytotoxicity, DPPH, phosphomolybdate

1. Introduction

Cleome is a genus that belongs to the family cleomaceae and consists of large number of species (Weli et al., 2021). The plants in this genus have unpleasant taste and smell, they are distributed in tropical and warm temperate regions (Sajjadi et al., 2018). Pharmacologically active compounds from *Cleome* species have played a significant role as curative agents in drug invention (Singh et al., 2018). The members of plant family Cleomaceae contain important bioactive compounds.

Cleome gynandra Linn. is a known medicinal plant with traditional and pharmacological importance. In Mubi, Adamawa State of Nigeria, concoction of the leaves of *C. gynandra* is used to treat typhoid and anaemia. The root is used as medicine for chest pain, while leaves extract is used to treat diarrhea, pneumonia and used as eyewash (Khan et al., 2015). Antioxidant (DeepaShree & Gopal, 2016, Bala et al., 2012), anti-cancer (Bala et al., 2010, Narendhirakannan et al., 2007) and anti-inflammatory effects (Saravanan et al., 2017) have also been confirmed from *C. gynandra*.

Plants contain several phytochemicals that possess strong antioxidant activities. An antioxidant is a substance that inhibits or delays oxidation of a specie even when present at low concentration (Antolovich et al., 2002). They are compounds capable to either delay or inhibit the oxidation processes that occur under the influence of atmospheric oxygen or reactive oxygen species (Pisoschi & Negulescu, 2011). Despite the proven therapeutic efficacy of plants, the possible toxic effect they might present is still unknown. The toxicity test on a nauplii of *Artemia salina* is a widely used method in natural products research to evaluate the toxic potential of plant extracts (Amado et al., 2018). This study is aimed at evaluating antioxidant and cytotoxic activities of *Cleome gynandra*.



2. Experimental

2.1 Plant material

Cleome gynandra whole plant was collected from Wase Dam, `Minjibir local government area, Kano State, Nigeria in January, 2017. Identification and authentication of the plant was carried out at the plant Biology Department, Bayero University Kano by Bahauddeen Adam. The sample was deposited in the departmental herbarium with voucher number BUKHAN 0408.

2.2 Extraction

Air dried powdered whole plant material was initially defatted using *n*-hexane (2 L), the residue was then extracted with ethanol (2 L) by cold percolation method for 10 days. The ethanol extract was partitioned using *n*-hexane, chloroform and 60% aqueous methanol gradient (Chanda & Baravalia 2011).

2.3 Free radical-scavenging activity

The free radical scavenging activity of the plant extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method described by Aicha *et al.* (2017) and Chandradevan *et al.*, 2020 with slight modification. Each sample of stock solution (20 mg/L) was diluted to final concentration of 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 µg/mL. Then, a total of 50 µM DPPH methanolic solution (160 µL) was added to sample solution (40 µL) and allowed to react at room temperature for 30 min in dark. The absorbance of the mixtures was measured at 517 nm. Ascorbic acid was used as positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. Inhibitions of DPPH radical in percent (I%) were calculated using the formula:

$$I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

Where,

A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and

A_{sample} is the absorbance values of the test compounds.

The sample concentration that provides 50% inhibition (IC_{50}) was determined using SPSS 16.

2.4 Total antioxidant activity by phosphomolybdenum assay

Total antioxidant activities of samples were determined according to the method of Prieto *et al.* (1999) with slight modification. An aliquot of 20 µL of sample solution (0.1 mg/mL) was mixed with 180 µL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min in a water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid. A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid and was expressed as mg ascorbic acid equivalents per gram of sample on a dry weight (DW) basis (Raja *et al.*, 2010).

2.5 Toxicity Assay

2.5.1 Hatching of brine shrimp

Brine shrimp eggs (Artemix, GmbH & Co., Germany), *Artemia salina* were hatched in distilled water (16 g in 500 mL). After 24 h incubation at room temperature, the larvae was attracted to one side of the vessel with a light source and collected by pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing seawater (Hamidi *et al.*, 2014).

2.5.2 Brine shrimp assay

Brine shrimp lethality test was used to monitor the toxicity of the extract according to the method described by Weli et al., 2021 and Olowa & Nuñez, 2013), with slight modification. 20 mg/ mL each of the extract was dissolved in methanol, from which 5000, 500 and 50 μL of each solution was transferred into vials corresponding to 1000, 100 and 10 $\mu\text{g}/\text{mL}$ respectively. The solution is allowed to evaporate to dryness for about 24 h at room temperature; Each dosage was tested in triplicate (9 per test sample). Seawater (4 mL) and 10 larvae were introduced into each vial. The final volume of solution in each vial was adjusted to 5 mL with seawater immediately after adding the shrimps. A negative control was prepared as a drug-free and potassium dichromate was used as positive control. Survivors were counted after 24 h, and LC_{50} was determined by probit analysis using SPSS version 16.

2.6 Data analysis

The experiments were performed in triplicate and results are presented as mean \pm SD (standard deviation). In DPPH and cytotoxicity study IC_{50} , LC_{50} values and 95% confidence intervals were determined using SPSS 16.

3. Results

Free radical scavenging activity of ethanol, n-hexane, chloroform and aqueous methanolextracts were evaluated and expressed as the percent inhibition as well as inhibitory concentration at 50% of the tested samples as presented in Figure 1 and Table 1 respectively. Antioxidant interact with and neutralize free radicals, thus preventing damage of cells from radicals (Iqbal et al., 2017)

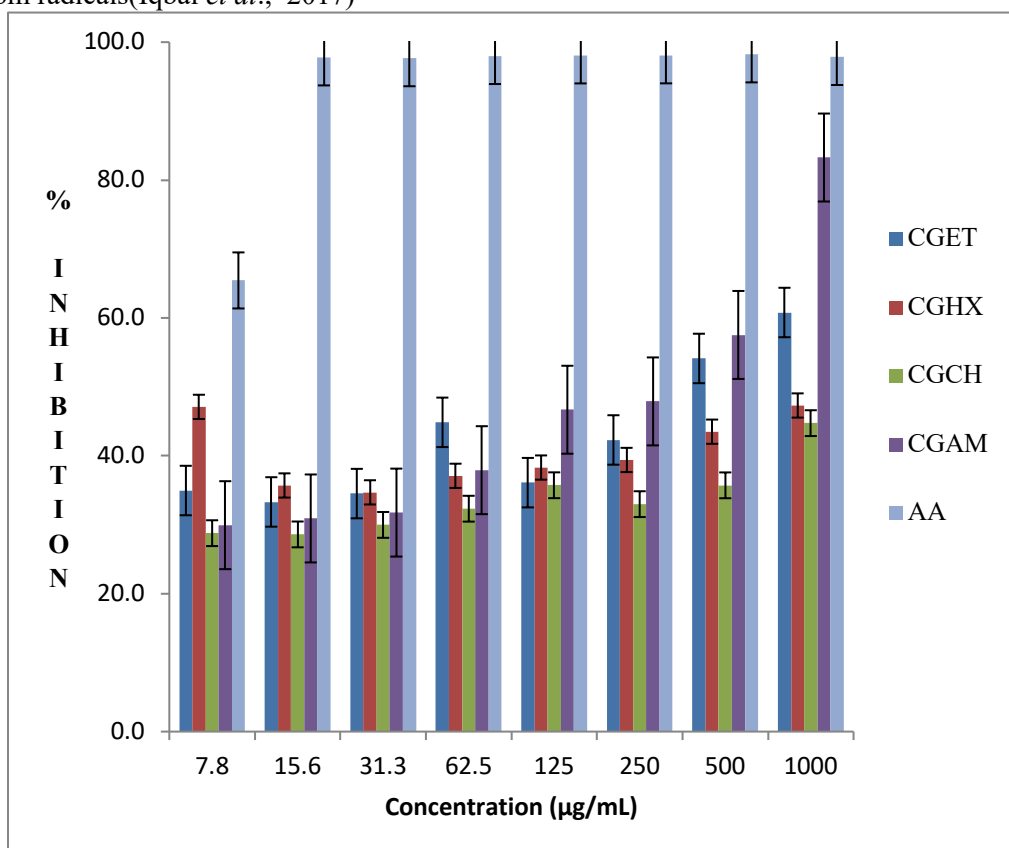


Figure 1. DPPH Percent inhibition of test samples

Key: CG: *C.gynandra*; ET: ethanol extract; HX: n-hexane fraction; CH: chloroform fraction; AM: aqueous methanol fraction; AA: ascorbic acid.

Table 1: Antioxidant Activities of *C.gynandra* Extracts

Sample	IC ₅₀ µg/mL
CGET	370.36
CGHX	>1000
CGCH	>1000
CGAM	137.43
AA	0.08

CG: *C.gynandra*; ET: ethanol extract; HX: *n*-hexane fraction; CH: chloroform fraction; AM: aqueous methanol fraction; AA: ascorbic acid.

Total antioxidant activities of the samples were also investigated using phospho-molybdate technique, ascorbic acid was used to estimate the antioxidant powers of the tested samples expressed as µg ascorbic acid equivalent per mg (µg AAE/mg) as presented in both Figure 2 and Table 2. Highest antioxidant power among the samples was observed in chloroform fraction with 38.23±0.05 µgAAE/mg, whereas lowest activity was exhibited by aqueous methanol fraction with estimation of 23.43±0.03 µg AAE/mg.

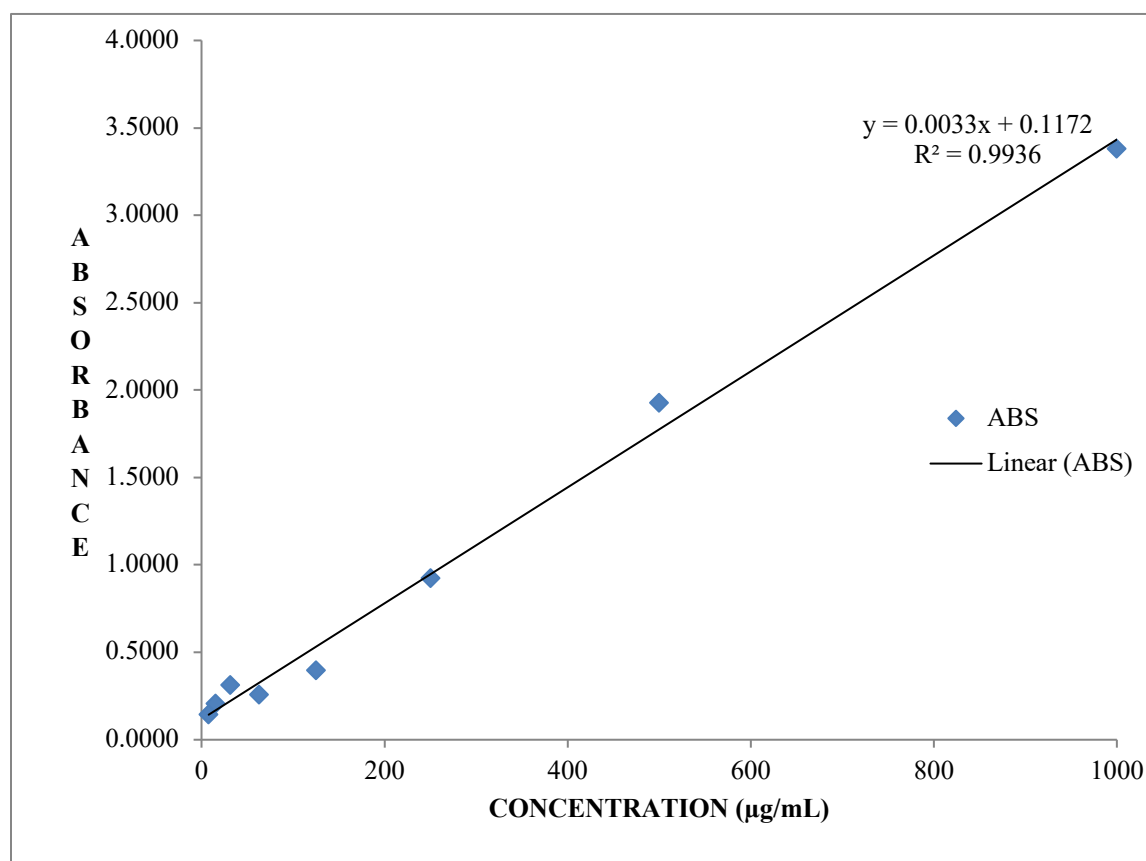


Figure 2: Ascorbic acid calibration curve

Table 2: Total antioxidant activity of the tested samples

Sample	Total antioxidant activity $\mu\text{g AAE/mg}$
CGET	29.97 \pm 0.03
CGHX	27.70 \pm 0.05
CGCH	38.23 \pm 0.05
CGAM	23.43 \pm 0.03

CG: *C.gynandra*; ET: ethanol extract; HX: n-hexane fraction; CH: chloroform fraction; AM: aqueous methanol fraction.

The toxicity of the extracts was expressed in $\mu\text{g/mL}$ as a concentration of the extract that will lead to the death of 50% (LC_{50}) of brine shrimp larvae tested. Table 3 indicated that, all the tested samples were found to be more toxic (7.20 – 76.09 $\mu\text{g/mL}$) than the positive control, potassium dichromate (87.36 $\mu\text{g/mL}$). In this study, *n*-hexane fraction of the plant was found to be the most toxic to brine shrimp nauplii, with LC_{50} of 7.198 $\mu\text{g/mL}$, this could be attributed to its oily nature that might prevent supply of atmospheric oxygen to the nauplii. This result is in agreement with the findings of Afifi (2014) who reported that *n*-hexane, chloroform and *n*-butanol fractions of *Cleome brachycarpa* showed significant brine shrimp lethalties with LC_{50} values of 15.0, 26.50, and 52.10 $\mu\text{g/mL}$ respectively.

Table 3: Toxicity of the *C.gynandra* extracts.

Sample	LC_{50} ($\mu\text{g/mL}$)
CGET	14.80
CGHX	7.20
CGCH	72.53
CGAM	76.09
$\text{K}_2\text{Cr}_2\text{O}_7$	87.36

CG: *C.gynandra*; ET: ethanol extract; HX: n-hexane fraction; CH: chloroform fraction; AM: aqueous methanol fraction; $\text{K}_2\text{Cr}_2\text{O}_7$: potassium dichromate.

4. Discussion

From figure 1, the scavenging activity of the tested samples at 1000 $\mu\text{g/mL}$ against DPPH radical followed the order of aqueous methanol fraction (83.3%), ethanol extract (60.8%), hexane fraction (47.7%), chloroform fraction (44.7%) lower than that of ascorbic acid (97.9%). It can be observed that the scavenging activity of the aqueous methanol fraction increased with increase in concentration, i.e a concentration-dependent manner. The 50% inhibitory concentration of the samples showed highest scavenging activity in the aqueous methanol fraction with IC_{50} value of 137.508 $\mu\text{g/mL}$ (Table 1). It has been reported that ethanol leaves extract of *Cleome Arabica* have a strong DPPH anti-radical activity with IC_{50} value of 0.09mg/ml as reported by Aicha *et al.*, (2017). In another study conducted by Widodo and Pratiwi (2018) where they investigated DPPH scavenging activity of ethanol whole plant extract of *C. gynandra* an inhibition with IC_{50} value of 189.46 $\mu\text{g/mL}$ was found to be 2 times more active than the observed value of similar extract with IC_{50} of 370.36 $\mu\text{g/mL}$ in the present study. This might be attributed to the geographical location or extraction technique.

Antioxidant activity by phosphomolybdate technique, involves an electron transfer (ET) by the reduction of molybdenum (VI) to molybdenum (V) in the presence of a reducing agent (antioxidant), with a formation of a green phosphomolybdate (V) complex that can be measured spectrophotometrically at 695 nm. Natural products such as phenols and flavonoids can cause this type of reduction (Ahmed *et al.*, 2015). The extracts from *C. gynandra* showed antioxidant activity in the order of chloroform>ethanol> n-hexane> aqueous methanol, indicating higher electron transfer in the chloroform fraction.

In this study, *n*-hexane fraction of the plant was found to be the most toxic to brine shrimp nauplii, with LC_{50} of 7.198 $\mu\text{g/mL}$, this could be attributed to its oily nature that might prevent supply of atmospheric oxygen to the



nauplii. This result is in agreement with the findings of Afifi (2014) who reported that *n*-hexane, chloroform and *n*-butanol fractions of *Cleome brachycarpa* showed significant brine shrimp lethality with LC₅₀ values of 15.0, 26.50, and 52.10 µg/mL respectively.

5. Conclusion

The extracts from the plant (*Cleome gynandra*) were evaluated for cytotoxicity, and antioxidant properties. The result obtained from this study revealed significant antioxidant activities of the plant extracts as well as their toxicity against brine shrimp larvae, suggesting their anti-tumor and anti-inflammatory potency of the plant as oxidative stress related diseases.

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