CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF *Boerhavia adscendens* Willd.

*A. KWAIJ, R. ATIKO and I. M. UMAR*

Department of Chemistry, Gombe State University, PMB 127 Gombe State, Nigeria.

Corresponding Author: andrewkwaji@yahoo.com

**ABSTRACT**

*Boerhavia adscendens* (*B. adscendens*) is a medicinal plant with widespread use in folk medicine. The present study was aimed at evaluation of the cytotoxicity and antioxidant activity of the crude ethanol extract of *B. adscendens*. The cytotoxicity of the extract was determined using brine shrimp lethality assay while its antioxidant activity was assessed using ferric reducing antioxidant potential (FRAP). The study revealed that the *B. adscendens* extract has moderate cytotoxic effect against the brine shrimp larvae with LC$_{50}$ of 100 μg/mL relative to K$_2$Cr$_2$O$_4$ standard with LC$_{50}$ of 0.01 μg/mL. The FRAP determination for *B. adscendens* extract yielded an IC$_{50}$ of 34.56 μg/mL while the ascorbic acid standard had IC$_{50}$ value of 31.25 μg/mL. These results demonstrate that the *B. adscendens* ethanol extract has moderate cytotoxicity and quite a significant antioxidant activity. Consequently, these findings may partly explain the usefulness of *B. adscendens* in traditional medicine.

**Key words:** Cytotoxicity, antioxidant, brine shrimp, *Boerhavia adscendens*

**INTRODUCTION**

The use of medicinal plants in healthcare around the globe dates back to ancient times for several reasons but not limited to lesser or benign side effects, better compatibility with human body (Partap *et al.*, 2012), affordability and efficacy (Razak *et al.*, 2011). The World Health Organization (WHO) estimates that about 4 billion people (ca 80% world population) use herbal medicines for their primary healthcare needs (WHO, 2002). Medicinal plants are rich in bioactive phytochemicals with broad application in medicine, food and beverage industries (Kontogianni *et al.*, 2013). *B. adscendens* is a wild tropical medicinal plant which can be found in Africa and Indian continents with several medicinal applications such as cardiotonic, hepatoprotective, laxative, diuretic, anti-dysentery, expectorant, antimalarial and anti-jaundice.

In Hausa, it is known as ‘Babban juji’. It is an annual herb that can grow to 1 m in height (Sheila *et al.*, 2013). Brine shrimp (*Artemia salina*) lethality assay is a preliminary test commonly used to screen for the cytotoxicity of bioactive phytochemicals of plant origin (Guérard *et al.*, 2015; Kibiti and Afolayan, 2016); It is simple, inexpensive and effective relative to other methods (Venkatesh *et al.*, 2013). Studies had shown that the consumption of plant diet high in phenolic content- is associated with low cardiovascular disorders risk and reduced cancer incidences (Altay and Bozoğlu, 2017). The medicinal properties of phenolic compounds had been attributed to their antioxidant property which protects cells against free radicals damage (Köksal *et al.*, 2017).

Antioxidants are of two types namely enzymatic such as glutathione peroxidase, catalase and superoxide dismutase and non-enzymatic antioxidants such as Vitamins E and C, melatonin, carotenoids and flavonoids (Mironczuk-Chodakowska *et al.*, 2018). Free radicals are continuously produced during cellular respiration and could precipitate
conditions such as Parkinson’s disease, Alzheimer’s disease, arthritis and diabetes if left unchecked (Singh et al., 2013). In a healthy body the amount of free radicals generated is regulated by its antioxidant defense systems (Adoum, 2009). These antioxidants can only protect when the amount of free radicals generated is within tolerable limits. Elevated levels of free radicals can cause oxidative stress with potential deleterious effects (Abbasi et al., 2012). In view of the critical importance of B. adscendens in ethnomedicine, there is need to evaluate its cytotoxicity index and antioxidant potential.

**MATERIALS AND METHODS**

**Plant Collection and Identification**

The plant was collected in October 2019 by a herbalist at Gombe old Market and identified by botanist at Department of Biological Sciences, Gombe State University, Nigeria. The plant sample was shredded to pieces, shade dried and coarsely grounded to powder. This was stored in an air tight container until required for use.

**Extraction and Preparation of Test Solutions**

The powdered plant material (1 kg) was soaked in 5 L of ethanol for a week with occasional shaking. The plant filtrate was obtained over a cotton plug in a funnel. It was re-filtered using whatmann No 1 filter paper. The filtrate was concentrated on a rotary evaporator at 45°C to yield the crude extract. A stock solution of concentration 100,000 μg/mL was prepared by dissolving 0.50 g of extract in 0.50 mL of DMSO and 4.50 mL brine solution or artificial seawater (ASW) to give a solution concentration of 100,000 μg/mL. The stock solution was diluted with ASW to produce working solutions of 5,000.00, 500.00 and 50.00 μg/mL respectively. Ten (10) shrimp larvae in 4.5 mL ASW were added to 0.5 mL of each plant extract working solution. Final concentration of test solutions were 1000, 100 and 10 μg/mL respectively (Suryawanshi et al., 2020).

**Brine Shrimp Test**

Artificial sea water (ASW) was prepared as described by Haque et al. (2014) by dissolving 38 g NaCl in de-ionized water and made up to a Litre of solution. The solution was adjusted to pH 8.5 with 1N NaOH and filtered to get clear solution. ASW was taken in a 500 mL beaker and Shrimp eggs were added to hatch and mature within 48 hrs. With the aid of a Pasteur pipette, 10 shrimp larvae in 4.5 mL ASW were added to test tubes each containing 0.5 mL of test solutions and Potassium dichromate (K₂Cr₂O₇) served as positive control (Suryawanshi et al., 2020). After 24 hrs of incubation, the test tubes were inspected using a magnifying glass and the number of survivors were counted. The percentage (%) mortality was calculated for each dilution. The median lethal concentration (LC₅₀) was determined graphically. This represents the concentration that produced death in half of larvae population after 18-24 hr exposure (Gautam et al., 2016).

**Ferric Reducing Antioxidant Potential (FRAP) Method**

The reducing power of B.adscendens crude extract was determined in accordance to method described by Yohanna et al. (2021) with ascorbic acid as the standard. About 1 mL of the extract and 1 mL of the standard at various concentrations (10, 20, 40 and 50 μg/mL) were mixed with 2.5 mL of Phosphate buffer (6.6 pH) and 2.5 mL of 1% K₃Fe(CN)₆. The mixtures were then incubated at 50 °C for 30 min. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid. The mixture was centrifuged at 3000 rpm for 10
min. About 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl₃ solution. Absorbances were read at 700 nm using UV-Vis Spectrophotometer. The fifty percent inhibitory concentration (IC₅₀) was evaluated graphically from a plot percentage Frap against concentration (Haque et al., 2014).

RESULTS AND DISCUSSION

Cytotoxic Assay of B. adscendens

The LC₅₀ value for B. adscendens crude ethanol extract was 100 μg/mL (Figure 1) while that of standard potassium dichromate gave an LC₅₀ of 0.01 μg/mL. The result indicates that the extract is moderately cytotoxic and hence connotes the presence of bioactive compounds (Suryawanshi et al., 2020). It also showed a higher cytotoxicity index compared to the LC₅₀ (165.19 μg/mL) value for the methanolic extract of B. diffusa which is a specie in the genus (Gautam et al., 2016). Similarly Khalid et al. (2011) also reported LC₅₀ value of the n-hexane extract of B. diffusa as 140.55 μg/mL. Notwithstanding, it is clear that the crude ethanol extract of B. adscendens exhibited a much higher LC₅₀ than those reported for B. diffusa crude extracts. The cytotoxic nature of these crude extracts strongly suggests the presence of bioactive compounds.

FRAP Assay

The fifty percent reducing capacity showed an IC₅₀ value of 34.56 μg/mL (Figure 2) for the ethanol extract which indicate a strong antioxidant potential. This is similar to that of the standard ascorbic acid with IC₅₀ of 31.25 μg/mL. The strong reducing power may be responsible for the efficacy and widespread use of B. adscendens in traditional medicine. The observed activity is much higher than that reported by Khalid et al. (2011) for the ethanolic extract of B. diffusa with maximum inhibition of DPPH free radical of 91.25% at concentration of 1.50 mg/mL and IC₅₀ value of 0.13 mg/mL. The screening of the hydro-alcoholic extract of B. diffusa using the DPPH model by Patel et al. (2014) revealed maximum percentage inhibition of 80% while the IC₅₀ value was 100 μg/mL. Ammar et al. (2014) reported the reducing capacity of ethanolic seed extract of B. elegans on DPPH free radicals. The IC₅₀ of the plant extract was 2.42 μg/mL while the ascorbic acid standard was 1.47 μg/mL. The two preceding examples illustrate how plant extract can exert strong antioxidant activity. Despite the existence of several studies on the antioxidant potentials of Boerhavia species, there are no reports on its ferric reducing antioxidant potential based on available literature. Consequently, this is the first time its Ferric Reducing antioxidant potential is reported.

![Figure 1: %Mortality of Boerhavia adscendens](image1)

![Figure 2: % Ferric Reducing capacity of B. adscendens](image2)
CONCLUSION

*B. adscendens* is a useful medicinal plant in Northern Nigeria with widespread application. The study had shown that *B. adscendens* possess moderate cytotoxicity and a strong antioxidant activity relative to the ascorbic acid standard. Therefore the study has laid credence to the usefulness of *B. adscendens* in ethnomedicine. It is also worthy of note that, this is first report on cytotoxicity and ferric reducing antioxidant potential of *B. adscendens* based on available literature reports.

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