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CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF *Boerhavia adscendens* Willd.

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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 $K_2Cr_2O_4$ 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^{o}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 ($K_2Cr_2O_4$) 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 (LC_{50}) 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% $K_3Fe(CN)_6$. 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% $FeCl_3$ solution. Absorbances were read at 700 nm using UV-Vis Spectrophotometer. The fifty percent inhibitory concentration (IC_{50}) was evaluated graphically from a plot percentage Frap against concentration (Haque *et al*., 2014).

RESULTS AND DISCUSSION

Cytotoxic Assay of *B. adscendens*

The LC_{50} value for *B.adscendens* crude ethanol extract was 100 µg/mL (Figure 1) while that of standard potassium dichromate gave an LC_{50} 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 LC_{50} (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 LC_{50} 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 LC_{50} than those reported for *B. diffusa* crude extracts. The cytotoxic nature of these crude extracts strongly suggests the presence of bioactive compounds.

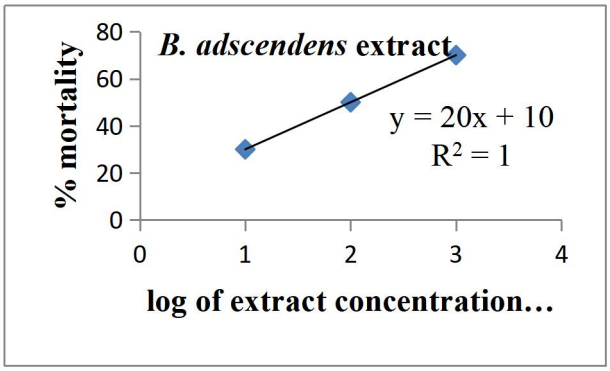

Figure 1: %Mortality of *Boerhavia adscendens*

FRAP Assay

The fifty percent reducing capacity showed an IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} value was 100 µg/mL. Ammar *et al*. (2014) reported the reducing capacity of ethanolic seed extract of *B. elegana* on DPPH free redicals. The IC_{50} 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.

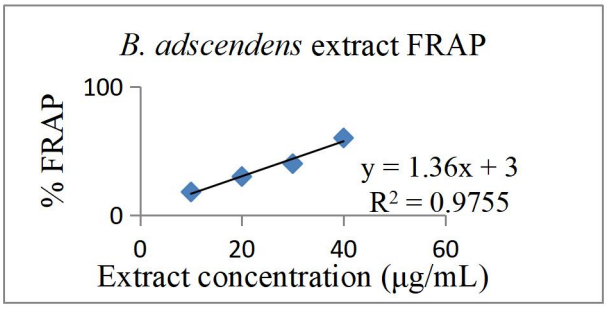

Figure 2: % Ferric Reducing capacity of *B. adscendens* .

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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