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RESEARCH ARTICLE

SCAVENGING EFFECT OF METHANOL CRUDE EXTRACT AND FRACTIONS OF *CRATEVA ADANSONII* LEAVES ON 1,1-DIPHENYL PICRYLHYDRAZYL RADICAL

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Glossary of Abbreviations:
CAE: *Crateva adansonii* extract
DPPH: Diphenyl picryl hydrazyl

ABSTRACT

Background: *Crateva adansonii* leaves have been used for many years traditionally in treating of headaches and inflammation. **Objective:** To determine the free radical scavenging effect of crude extract and fractions of *Crateva adansonii* leaves. **Methods:** The free radical scavenging activities of the methanol crude extract and fractions of the plant leaves were investigated in this study using 1,1-diphenyl picryl hydrazyl (DPPH) photometric assay. **Results:** The percentage yield of the leaves was 13.87 w/w. 35g of the crude methanol extract was subjected to solvent-solvent partitioning to yield 7.5 g, 2.0 g, and 3.1 g and 8.56 g of n-hexane, chloroform, ethyl acetate and methanol fractions respectively. All fractions showed dose-dependent increases in antioxidant activity. However, ethyl acetate fraction had the highest anti-oxidant activity, with mean percentage antioxidant value of 97.58±0.03 and 98.55±0.12 at 200 and 400 µg/ml respectively which was higher than that of ascorbic acid, 95.62±0.22 and 96.67±0.07 at 200 and 400 µg/ml respectively. **Conclusions:** The high anti-oxidant activities of the crude extract and fractions of the plant could be responsible for its wide use as an anti-inflammatory therapy in ethno-medicine.

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INTRODUCTION

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent and oxidants are highly reactive oxygen molecules that are capable of becoming part of potentially harmful molecules generally called 'free radicals' (Bland, 1995). Oxidation reactions produce free radicals, which cause damage or cell death. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function and have been implicated in the pathogenesis of many diseases. Cell damage caused by free radicals appear to be a major contributor to ageing and to degenerative diseases including cancer, inflammation, cardiovascular disease, cataracts, immune system dysfunction and brain damage (Benzie, 1999). Several epidemiological studies have shown that compounds that can scavenge free radicals (antioxidants) are effective in ameliorating the progress of these related diseases.

Antioxidants are defined as naturally occurring bioactive molecules that inhibit the process of oxidation even at relatively small concentrations and thus elicit diverse physiological roles in the biological systems. They act as free radical scavengers, convert free radicals to less reactive species and defend against oxidative damages (Phaniendra *et al*., 2015). However, there has been growing concern over the safety and toxicity of synthetic antioxidants recently (Bhoyar, 2011). Therefore, naturally occurring antioxidants, because of lack of toxicity and adverse effects are presently attracting more attention (Hongmei, 2011). *Crateva adansonii* is a deciduous tree; usually grows to 3-10 metres tall. The leaves are trifoliate and ovate to oblong in shape. The flowers are white or creamy; it is widely distributed in Africa, from Senegal to Northern Nigeria and across to Zaire, Tanganyika and Madagascar. In Nigeria, it is called 'unguduudu' in Hausa, 'egun orun' in Yoruba and 'amakarode' in Igbo (Burkill,

1994). In ethno-medicine, the plant is used in treating swellings and inflammatory conditions, asthma, snakebites and as astringent (Akanji *et al*., 2013). Phenolics, alkaloids, flavonoids and saponins have been reported to be present in the leaves (Abdullahi *et al*., 2012). *Cratevaadansonii* stem bark has been reported to possess analgesic activity (Ndarubu *et al*., 2016). Although its antioxidant activity has been established in previous studies, these were only conducted on the crude extract (Abdullahi *et al*., 2012). There are no reports on the antioxidant activities of fractions of *C. adansonii* leaves to the best of our knowledge. This study is aimed therefore, at studying the anti-oxidant activity of crude extracts and fractions of the leaves of *C. adansonii* using the 2, 2-diphenyl picryl hyrdrazine photometric model.

MATERIALS AND METHODS

Plant identification and extraction: Fresh leaves of *Crateva adansonii* were collected in November, 2015 at Orba in Udenu Local Government Area of Enugu State, Nigeria. The plant was identified by Mr. Ozioko, a botanist. The leaves were air-dried at room temperature, ground using a manual blender and extracted in 80 % methanol by cold maceration for 48 h, with intermittent shaking every 2 h. This was filtered using What man's filter paper. The filtrate was dried in the hot air oven at 40 ºC and stored in the refrigerator at 4 ºC as *Crateva adansonii* extract (CAE) until further use. Percentage yield was calculated as follows

$$\%\ YIELD = \frac{b}{a}\ x\ \frac{100}{1}$$

Where a = Weight of original plant material used for extraction and b = weight of the recovered extract.

Solvent-solvent partitioning: Thirty-five grammes (35 g) of the CAE was dissolved in 40 % aqueous methanol. This was successively partitioned using n-hexane, chloroform, ethyl acetate and methanol in order of increasing polarity using a separating funnel. The fractions or partitions were collected in separate conical flasks. These fractions were dried in the hot air oven at 40 ºC and later refrigerated at 4 ºC for future use (Udeh, 2015).

Evaluation of *invitro* antioxidant activity of CAE using 1-1 Diphenyl-2 picrylhydrazyl (DPPH) photometric assay: The free radical scavenging activity of the extract was evaluated by DPPH photometric assay using the method described by Mensor *et al.,* (2011). Two (2) ml of CAE and each of the fractions at different concentration (25, 50, 100, 200 and 400 µg/ml) each were mixed with 1.0 ml of 0.5 mM DPPH in methanol in a cuvette. The absorbance of the resulting solution was read at 517 nm after 30 min of incubation in the dark at room temperature with a spectrophotometer. All concentrations were prepared in triplicates. The percentage antioxidant activity was calculated as follows using mean values.

% Antioxidant Activity = ({(Ab_{sample} – Ab_{blank}) x 100)}/$Ab_{control}$)

Where, Ab_{sample} is the absorbance of sample
Ab_{blank} is the absorbance of blank
$Ab_{control}$ is the absorbance of control

Methanol (1 ml) plus 2 ml of the extract was used as the blank while a mixture of 1ml of 0.5 mM DPPH solution plus 2.0 ml of methanol was used as negative control. Ascorbic acid (1ml) in 1 ml DPPH was used as reference standard antioxidant.

RESULTS

The methanol fraction of *Crateva adansonii* had the highest yield of 24. 45 % w/w (Table 1). The anti-oxidant activity of the extract compared to the standard drug is presented in Figure 2. The extract exhibited a dose-dependent increase in free radical scavenging activity from 2.22 % at 25 µg/ml to 86.76 % at 400 µg/ml. Ascorbic acid had better activities at these concentrations. All the fractions of CAE had dose-dependent anti-oxidant activity in DPPH photometric assay. However, ethyl acetate fraction exhibited the highest activity of 96.66 %, 97.58 % and 98.55 % at 100, 200 and 400 µg/ml. These were better than that of the ascorbic acid which had 95.58 %, 95.62 % and 96.07 % at these concentrations (Fig. 2).

Table 1. Weights and percentage yields of crude extract and fractions of CAE

Extract/fraction	Amount(g)	Percentage yield (%)
Crude	69.09	13.87
Methanol fraction	8.56	24.45
Ethylacetate	3.10	8.85
Chloroform	2.00	5.71
n-hexane	7.50	21.42

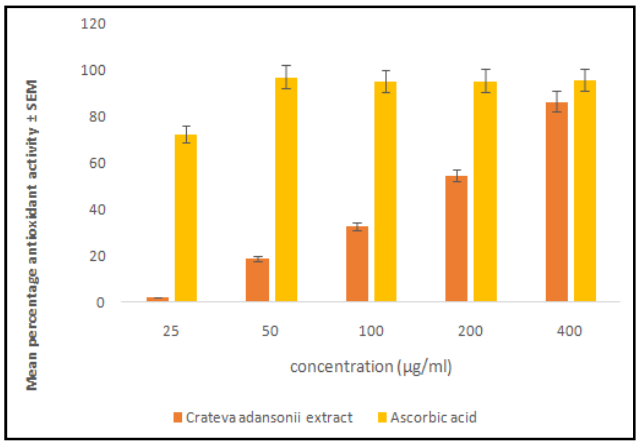

Figure. 1. Percent antioxidant activity of CAE in DPPH assay

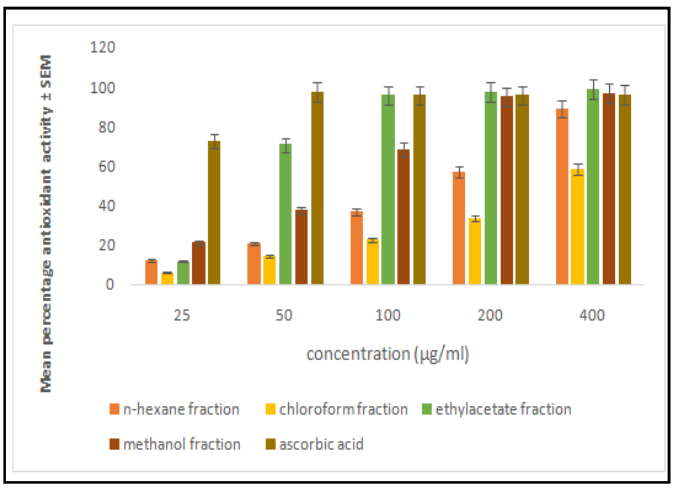

Fig. 2. Percent antioxidant activity of *Crateva adansonii* fractions in DPPH assay

DISCUSSION

In this study, free radical scavenging activity of CAE was assessed using 1,1-diphenyl picrylhydrazyl (DPPH) photometric assay. This method was developed by Blois (1958) with the viewpoint to determine the antioxidant activity in a like manner, but by using a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M = 394.33). The assay is based on the measurement of the scavenging capacity of antioxidants towards DPPH. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Mensor *et al*., 2001). The extract and fractions all exhibited some level of anti-oxidant activity. However, the ethyl acetate fraction had higher free radical scavenging activity than the crude extract and other fractions and was better than the standard drug. The anti-oxidant activity of crude extract of *Crateva adansonii* has been previously reported. However, there is lack of information on the free radical scavenging activity of the fractions of this plant. Flavonoids are natural polyphenolic molecules of plant origin known for their antioxidant, anti-inflammatory and anti-carcinogenic properties (Blois, 1958). This class of phytochemicals are usually found in the methanol and ethylacetate fractions of plants and have been widely reported in *Crateva adansonii* extract. The anti-oxidant activity of flavonoids has been attributed mainly to their capacity to scavenge oxygen and reactive nitrogen species (Kedare, 2011) and to chelate redox-active metals (Pinent, 2008). Thus, the free radical scavenging activity seen in the crude and ethylacetate fraction of this plant could be attributable to flavonoid content reported in previous studies. More studies are however required to isolate the free radical scavenging compounds in the ethylacetate fraction of *Crateva adansonii* leaves.

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