In-vitro* antioxidant screening of ethanol extracts of *Costus afer* and *Justicia carnea leaves

ABSTRACT

This study evaluated the *in-vitro* antioxidant activity of ethanol extracts of *Costus afer* and *Justicia carnea* leaves. Ethanol extracts of the plant leaves were obtained using standard procedures. The antioxidant parameters of the plants extracts studied were 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Ferric Reducing Antioxidant Power (FRAP) and Nitric oxide. In each case, the result of test was compared with that of a standard Ascorbic Acid (Vitamin C). The antioxidant study showed that *Justicia carnea* extract had a significantly higher Nitric oxide radical scavenging ability (04.16 ± 0.68) compared with *Costus afer* (02.15 ± 0.26) at 50 µg/ml concentrations while DPPH scavenging ability of the extracts of *Justicia carnea* showed no significant difference with *Costus afer* (07.63 ± 0.42) and (06.29 ± 0.53) at 50 µg/ml concentrations respectively. In case of FRAP test, it showed that ethanol extract of *Costus afer* (01.78 ± 0.22) was significantly lower ($p<0.05$) than that of *Justicia carnea* (05.12 ± 0.22) at 800 µg/ml concentrations. The results suggest that *Costus afer* and *Justicia carnea* ethanol extracts could serve as free radical scavengers, acting as primary antioxidants. The results support local claims of their therapeutic uses in the treatment of malaria in folklore medicine.

Keywords: Antioxidants, *Costus afer*, *Justicia carnea*, antioxidant, medicinal plants

INTRODUCTION

Plants and its derived natural products have received considerable attentions recently because of its pharmacological properties such as antioxidant activity (Karthikumar et al., 2007). Medicinal herbs have therefore become of interest due to their prospects in meeting the health needs of mankind. (Ameh *et al*., 2010 and Ige *et al*., 2012). Among all components used in battling chronic diseases, phytochemicals, plant derived molecules endowed with steady antioxidant power have been in the forefront. The cumulative and synergistic activities of the bioactive molecules present in plant food have been reported to be responsible for their enhanced antioxidant properties (Abdalla, 2009).

Oxidative damage to proteins and nucleic acids gives rise to a variety of specific damage products as a result of modifications of amino acids or nucleotides (Sataro and Zsolt, 2013). Such oxidative damage might lead to cellular dysfunction, and it is this that might contribute to the pathophysiology of a wide variety of diseases.
In Nigeria and many African countries, herbs and leafy vegetables are used as food, food drinks and for medicinal purposes (Nwaogu et al., 2007). The use of herbs requires good knowledge of the toxicity, dosage purity, and suitable extraction solvent and adverse effects for effective usage (Paulo et al., 1994; Murray, 1998).
It is known as "Okpete" in Igbo, in Hausa "tete-egun" "Kakizawa" in Yoruba and "Mbritem" in Efik all in Nigeria.(Oliver, 1960). This plant is used in the treatment of inflammation, arthritis, as laxative, as purgative, diuretic, in rheumatism and treatment of several other diseases (Awouters et. al. 1978). Synthetic drugs are used in the treatment of disease but because of the high cost and side effects associated with their use (Chattopadhyay and Bandyopadhyay, 2005), attention is now directed towards the use of medicinal plant products in the prevention or management of most diseases.

Justicia carnea (Flamingo plant) is a flowering plant of Justicia, belonging to the *Acanthaceae* family (Corrêa and Alcântara, 2012). It widely distributed in various parts of Africa. In Nigeria, the shrubs are grown around homesteads and act as fences. *Justicia carnea* is called "hospital too far" in some parts of Nigeria while others refer to it as "ogwu obara" meaning blood tonic. Traditionally, several species of *Justicia* are used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes,diarrhea, liver diseases, rheumatism and arthritis (Badami *et al*., 2003; Corrêa and Alcântara, 2012). Phytochemical analysis of leaves crude extracts of *Costus afer* and *Justicia carnea* has revealed the presence of flavonoids, saponins, alkaloids, tannins, phenols and glycosides (Iwu *et al.*, 2009; Anaga *et al.*, 2004).

The aim of this study is to determine the scientific bases for the use of *costus afer* and *justicia carnea* by evaluating its *in-vitro* antioxidant activities. This is important because of the increasing demand for medicinal plants and plant products as alternatives to orthodox medicines especially in developing nations.

MATERIALS AND METHODS

PLANT COLLECTION

The leaves of *C. afer* were harvested at Ihiagwa, Owerri West Local Government Area, Imo State, while leaves of *J. carnea* were harvested at Umuezeala, Eziobodo in Owerri West Local Government Area, Imo state Nigeria.

PLANT IDENTIFICATION

The fresh leaves were identified by Prof. D. I. Edet of the Department of Forestry and Wildlife Technology, School of Agriculture and Agricultural Technology (SAAT), FUTO. The plants were authenticated by another taxonomist Dr. F. A. Faruwa of the Department of forestry and wildlife technology, SAAT, FUTO. The leaves of *C. afer* were prepared and kept at herbarium with voucher number FUTO/FWT/HERB/2019/056, and for *J.carnea* FUTO/FWT/HERB/2019/057.

PLANT EXTRACTION

The plant leaves were harvested in large quantities and then thoroughly washed to get rid of unwanted particles before air-dried at room temperature (27° C- 31°C) for about one (1) month to constant weight under shade. The dried samples were pulverized into powdered form using a diesel powered grinder and then stored separately in an air-tight containment. A quantity of 300g of each powdered sample was soaked separately in 1800ml of absolute ethanol of analytical grade, for 72 hours. Each sample solution was filtered. The filtrates were separately concentrated using water-bath at temperature of 45°C. All extracts were weighed and then stored in well stoppered containers and preserved in refrigerator maintained at a temperature of 4°C until subsequent use.

LABORATORY ANALYSIS

The antioxidant parameters of the plants extracts analyzed by spectrophotometric methods were 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical, Ferric Reducing Antioxidant Power (FRAP) and Nitric oxide. In each case result of test was compared with that of a standard Ascorbic Acid (Vitamin C).

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

DPPH radical scavenging activity of the samples was estimated as described by Mensor et al., 2001. The crude extract at concentrations (50,100, 200, 400, and 800) µg/ml each was mixed with 1ml of 0.5 mM DPPH (in ethanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activities were calculated as follows:

% Antioxidant activity (AA) = 100- [{ Sample – Blank) ×100}/ control]
One milliliter of methanol plus 2.0 ml of the test extract was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid (Vitamin C) was used as reference standard.

REDUCING POWER ASSAY
The reducing property of the samples was determined as described by Benzie and strain (1996). FRAP working solution was prepared by mixing Acetate buffer (300 mM) at pH 3.6 (3.1 g sodium acetate. $3H_20$ and 16ml glacial acetic acid in 1000 ml buffer solution) as solution 1, and then 2, 4, 6-triphridyl-s-triazine (TPTZ) (10 mM) in 40 mM HCL as solution 2 and finally $FeCl_3$ $6H_20$ (20 mM) in distilled water as solution 3.
FRAP working solutions was prepared by mixing solution 1, 2, and 3 in the ratio of 10:1:1, respectively. The working solutions were freshly prepared in each test. The aqueous solution of known amount of ascorbic acid was used as calibration. Blank; FRAP reagent. Sample; FRAP reagent (3ml) and 100 µl sample solution at concentrations of 50, 100, 200, 400, and 800 µg/ml were mixed and allowed to stand for 4 minutes. Colometric readings were recorded at 593 nm, at 37 ºC. The ascorbic acid standard solution was tested in a parallel process. Calculations were made by a calibration curve.

NITRIC OXIDE SCAVENGING ACTIVITY ASSAY
Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which were measured by Griess reaction. The reaction mixture (3ml) containing sodium nitroprusside (10mM) in phosphate buffer saline (PBS) and the extract from (50 – 800) µg/ml was incubated at 25ºC for 15 minutes. After incubation, 0.5 ml of the reaction mixture was removed and 0.5ml of Griess reagent (1% (w/v) sulfanilamide, 2% (w/v) $H_3PO_4$and 0.1% (w/v) naphthylethylenediamine hydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm.

Statistical Analysis

Statistical analysis was carried out with the aid of IBM SPSS statistics for windows; SPSS Inc., Chicago, Standard version 20 to determine differences between the mean of the tests. Post-hoc analysis was also performed to deduce the level of significant differences between the variables. All analyses were performed in triplicate. Data obtained was analyzed using multiple analysis of variance

(MANOVA) and the results were expressed as mean ± standard deviation. $P < 0.05$ was considered significant.

RESULT

Table1: Percentage (%) inhibition towards DPPH free radicals

Concentration (µg/ml)	**Activities of *J.carnea***	**Activities of *C. afer***	**Activities of Ascorbic acid**
50	07.63 ± 0.42^{a}	06.29 ± 0.53^{a}	42.02 ± 0.45^{b}
100	48.93 ± 0.86^{b}	21.91 ± 1.57^{a}	68.94 ± 0.74^{c}
200	52.88 ± 0.56^{b}	29.95 ± 0.72^{a}	80.16 ± 1.68^{c}
400	74.58 ± 0.89^{b}	60.26 ± 1.63^{a}	83.18 ± 0.91^{c}
800	87.93 ± 0.87^{b}	80.38 ± 0.88^{a}	92.61 ± 0.58^{c}

The analysis was carried out in triplicates and the results presented as mean ± standard deviation. The row bearing different superscripts are statistically different at $p < 0.05$.

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Table 2: Percentage (%) reducing power of *Justicia carnea* and *Costus afer towards FRAP*

Concentration (µg/ml)	**Activities of *J. carnea***	**Activities of *C. afer***	**Activities of Ascorbic acid**
50	00.10 ± 0.01^{b}	00.00 ± 0.00^{a}	00.39 ± 0.02^{c}
100	00.44 ± 0.03^{b}	00.04 ± 0.03^{a}	00.63 ± 0.01^{c}
200	01.35 ± 0.07^{b}	00.54 ± 0.04^{a}	03.53 ± 0.13^{c}
400	02.12 ± 0.18^{b}	00.92 ± 0.03^{a}	04.58 ± 0.42^{c}
800	05.12 ± 0.22^{b}	01.78 ± 0.02^{a}	06.94 ± 0.24^{c}

The analysis was carried out in triplicates and the results presented as mean ± standard deviation. The row bearing different superscripts are statistically different at $p < 0.05$.

Table 3: Percentage (%) inhibition towards nitric oxide radicals

Concentration (µg/ml)	**Activities of *J. carnea***	**Activities of *C. afer***	**Activities of Ascorbic acid**
50	04.16 ± 0.68^{b}	02.15 ± 0.26^{a}	05.91 ± 0.12^{b}
100	21.31 ± 1.29^{b}	04.82 ± 0.17^{a}	25.26 ± 0.46^{c}
200	36.11 ± 1.12^{b}	19.29 ± 0.73^{a}	60.89 ± 0.63^{c}
400	46.69 ± 0.61^{b}	31.91 ± 1.74^{a}	79.21 ± 0.76^{c}
800	79.04 ± 1.30^{b}	50.47 ± 1.23^{a}	90.16 ± 0.53^{c}

The analysis was carried out in triplicates and the results presented as mean ± standard deviation. The row bearing different superscripts are statistically different at $p < 0.05$.

DISCUSSION

Medicinal value of plants lies in some chemical substances that produces definite physiological action on the human body (Hussain *et al*., 2018). There is ample evidence to support the health benefits of medicinal plants (Awuchi, *2019*). Because plants contain complex mixtures of bioactive compounds, information

on the potential health of individual phytochemical is linked to information on the health effects of plants that contain those phytochemicals (Ahmad *et al*., 2018).

Several research works have been carried out on countless plants and they have received great attention because they contain high amounts of known antioxidants such as polyphenols, vitamin C etc. The consumption of these plants has been reported to be inversely associated with morbidity and mortality from degenerative diseases (Rodriguez and Costa, 2006).

The result of radical scavenging activity of the samples towards stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical as analyzed against the reference antioxidant ascorbic acid is shown in Table 1. It clearly shows that as concentration increases from 50 µg/ml to 800 µg/ml, samples exhibited increasing scavenging activity for both *Costus afer* and *Justicia carnea. results further revealed* the highest scavenging activity of 87.93 ± 0.87 % at a concentration of 800 µg/ml for *Justicia carnea*, while *Costus afer* showed the highest scavenging activity of 80.38 ± 0.88% at the same concentration.

It has been reported that *Costus afer* and *Justicia carnea* are rich sources of flavonoids and other phenolics (Anyasor *et al*., 2013; Ukpabi *et al*., 2012; Correa and Alcantara, 2012). The cumulative and synergistic activities of the bioactive molecules present in medicinal plants have also been reported to be responsible for their enhanced antioxidant properties (Hermali *et al.*, 2016).
DPPH radical is known to be used as the model system to investigate the scavenging activities of most natural compounds (Bhaskar *et al*., 2007). DPPH is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by the decreased absorbance. (Houcine *et al*., 2017). The high DPPH scavenging activity of the plant extracts recorded in this study would be attributed to the high phytochemical constituents. This is in line with various studies on scavenging abilities of flavonoids (Okawa *et al*., 2001; Zhang *et al*., 2012)

Reducing power of any compound can be used as indicator of its ability to serve as antioxidant (Zhiyong and Yuanzong, 2004), it acts by donating hydrogen that subsequently stabilizes free radicals. (Satish and Dilipkumar, 2015). The present study indicated varying reducing capacity that trail that of DPPH scavenging assay. *Justicia carnea* showed the best antioxidant property with regards to reducing power of 05.12 ± 0.22% at a concentration of 800 µg/ml, while *Costus afer* did not indicate any noticeable antioxidant property with regards to reducing power at 50 µg/ml. The consistent high value of *Justicia carnea* and its closeness to the values

of ascorbic acid which was used as a reference antioxidant suggests that is has the best antioxidant property with regards to reducing power. This property can be attributed to the presence of important biopharmaceutical phytochemicals.

Nitric Oxide is involved in the mediation of important physiological activities like regulation of cellular toxicity (Katia *et al*., 2011). The result indicated that nitric oxide scavenging activity varies with the concentration. As concentration increases from 50 µg/ml to 800 µg/ml, samples exhibit increasing activity for both *Costus afer* and *Justicia carnea. Justicia carnea* showed the highest scavenging activity of 79.04 ± 1.30% at a concentration of 800 µg/ml, while *Costus afer* showed the lowest scavenging activity of 02.15± 0.26 %.

CONCLUSION

The leaves extracts of *Justicia carnea* and *Costus afer* have been widely used in folklore medicine for the treatment of various sickness. This study serves as scientific proof of its pharmacological activities and link to its antioxidant capability. The strong antioxidant potential exhibited by these plants extracts shows that the plant could be used in the treatment and management of diseases arising from oxidative damage. Further study is however needed on possible toxic effect on chronic usage.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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