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 extract of *Costus afer* and *Justicia carnea* leaves. Ethanol extract of the plant leaves were obtained using standard procedures. The antioxidant parameters of the plants extract 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 concentration while DPPH scavenging ability of the extract 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. For of FRAP test, the result indicated 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 concentration. The findings show that ethanol extracts of Costus afer and Justicia carnea could operate as main antioxidants and free radical scavengers. The findings back up local assertions that they can be used to treat 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 at 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 damaged 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 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, asa laxative, 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 is 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).

This study aims to determine the scientific bases for the use of *costus afer* and *Justicia carnea* by evaluating their *in-vitro* antioxidant activities. This is significant because, particularly in developing countries, there is a growing desire for medical plants and plant products as alternatives to orthodox medicines.

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 the 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 being 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 the 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 a water-bath at a temperature of 45 °C. All extracts were weighed and then stored in well-stoppered containers and preserved in a refrigerator maintained at a temperature of 4°C until subsequent use.

LABORATORY ANALYSIS

The antioxidant parameters of the plant 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 the 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 of 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 the 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 were prepared by mixing solution 1, 2, and 3 in the ratio of 10:1:1, respectively. The working solutions were freshly prepared for each test. For calibration, an aqueous solution containing a known quantity of ascorbic acid was utilized. FRAP reagent; blank Sample; 50, 100, 200, 400, and 800 g/ml concentrations of FRAP reagent (3ml) and 100 l sample solution were mixed and left to stand for 4 minutes. At 37˚c, coulometric values were taken at 593 nm. A parallel procedure was used to test the ascorbic acid standard solution. A calibration curve was used to make the calculations.

NITRIC OXIDE SCAVENGING ACTIVITY ASSAY
Nitric oxide reacts with oxygen in an aqueous solution at physiological pH to form nitrite ions, which can be quantified using the Griess reaction. The reaction mixture (3ml) containing sodium nitroprusside (10mM) in phosphate buffer saline (PBS) and the extract from (50 – 800) µg/ml were 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) naphthyl ethylenediamine 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 toward 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 were presented as mean ± standard deviation. The rows bearing different superscripts are statistically different at $p < 0.05$.

Table 2: Percentage (%) reducing power of *Justicia carnea* and *Costus afer 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 were presented as mean ± standard deviation. The rows bearing different superscripts are statistically different at $p < 0.05$.

Table 3: Percentage (%) inhibition toward 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 were presented as mean ± standard deviation. The rows bearing different superscripts are statistically different at $p < 0.05$.

DISCUSSION

"The medicinal value of plants lies in some chemical substances that produce 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).

"These phytochemicals are known to possess a variety of biological activities including antimicrobial, antioxidant, anti-inflammatory and anticancer activities" (Ndukwe *et. al*., 2020).

"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). Another study by Jack *et al*. (2020) reported that "the reducing power of plants correlates with its phenolic content".

There might be possible toxic effect of chronic usage of these extracts. The results of the study by Kalu et. al., 2020 suggested that chronic administration of ethanol extract of *Ficus capensis* at 250 mg/kg and 150 mg/kg may induce some level of

anemia and may lead to infection, due to the presence of contaminants in such leaves at a higher dosage.
Table 1 shows the results of the samples' radical scavenging activity against the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical as compared to the reference antioxidant ascorbic acid. 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 the scavenging abilities of flavonoids" (Okawa *et al*., 2001; Zhang *et al*., 2012)

"The reducing power of any compound can be used as an indicator of its ability to serve as an 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 the 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 it 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 the regulation of cellular toxicity (Katia *et al*., 2011). The result indicated that

nitric oxide scavenging activity varies with 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

This study serves as scientific proof of the use of *Justicia carnea* and *Costus afer* in folklore medicine for treatment of various sicknesses. The leave extracts exhibited significant pharmacological activities that serve as a link to its antioxidant capability. However, more research on the potential toxicity of long-term use is required.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is 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 the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors

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