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Leaf extract of *Justicia carnea* is a potential *in-vitro* free radical scavenger

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ABSTRACT

Justicia carnea is a plant of the Acanthacea family that thrives in tropical regions of the world including Nigeria. Local herbal practitioners in Southeast Nigeria claim that the leaves of this plant, when soaked in high alcoholic liquid, are used against many inflammatory infections. In this study, standard method was employed ininvestigating the antioxidant potentialsin the methanol extract of*Justicia carnea* leaves.Results of the study showed thatthe methanol extract of *Justicia carnea* leaves reported a percentage RSA of 0, 50.46, 85.19, 86.64, 86.04. 88.24 according to their different concentrations which are 0, 200, 400, 600, 800, 1000 µg/ml respectively.Comparing these values with that of BHA which was the standard free radical agent used, *Justicia carnea*gavepercentage RSA values close to that of the standard. This result confirmed that*Justicia carnea*has strong antioxidative ability to reduce DPPH radical to the non-radical form. Results further showed that the EC_{50} for *Justicia carnea* gave 544.87µg/ml while that of BHA was 739.78. This result confirmed *Justicia carnea* leaves to have antioxidative ability and therefore prevent oxidative stress caused by free radicals.

Keywords: *Justicia carnea*, Free radicals, Scavenging properties, Antioxidants, DPPH

INTRODUCTION

Recent studies have shown that many diseases are due to oxidative stress resulting from imbalance between formation and neutralization of peroxidants [1,2].Oxidative stress is initiated by free radicals which seek stability through election pairing with biological macromolecules such as proteins, lipids and DNA [3,4]. This leads to damage to these biomolecules for example protein denaturation, DNA degradation and lipid peroxidation [5,6]. These changes contribute to cancer,atherosclerosis, cardiovascular, inflammatory diseases and ageing [7,8] and the preservation of the integrity of these molecules is essential for optimum health. Human cells protect themselves against free radical damage by enzymatic antioxidants such as superoxide dismutase (SOD) and catalase, or non-enzymatic ones, such as ascorbic acid, tocopherol and glutathione [9]. Sometimes these protective mechanisms one disrupted by various pathological processes and antioxidant supplements may be vital to combat oxidative damage [10].

Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation [11]. Free radicals can damage cells and may play a role in heart disease, cancer and other diseases [12,13,14,15]. Studies suggest that a diet high in antioxidants from fruits and vegetables is associated with a lower risk of cancer, cardiovascular disease, Parkinson's disease and Alzheimer's disease [16]. Plant-based diets protect against chronic oxidative stress-related diseases. Dietary plants contain variable chemicals that act as antioxidants. It has been hypothesized that plant antioxidants may contribute to the beneficial effects of dietary plants. Various studies have demonstrated that phytochemicals in common fruits and vegetables can have complementary and over lapping

mechanism of action, including scavenging of oxidative agents, stimulation of immune system, hormone metabolism and antibacterial and anti- viral effects [17].Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties [12].

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.

AIMS AND OBJECTIVES OF STUDY

- The aim of this work is to determine the nutrients in plants that are beneficial to man.

The objectives of this study will focus on revealing:

- DPPH free radical scavenging activity of *Justicia carnea* leaves.
- Presence of bioactive components in *Justicia carnea*leaves.
- The concentration at which the plant extract scavenges maximally.
- The EC_{50} of methanol extract of *Justicia carnea* leaves.

MATERIALS AND METHODS

METHODS

SAMPLE COLLECTION, IDENTIFICATION ANDPREPARATION

Justicia carnea leaves were harvested from the Enugu State University of Science and Technology(ESUT) environs about 3pm. Harvested leaves were identified by Prof Eze of the Applied Biology and Biotechnology Department of ESUT.

Identified leaves were air-dried and later ground to fine particles which were used for the study.

SAMPLE EXTRACTION

A weighed quantity of 20g of the ground *Justiciacarnea* leaves were weighed out using a weighing balance. The sample was poured into a conical flask; 200ml of methanol was measured out using a measuring cylinder and poured into the conical flask containing the sample. Extract from this sample was prepared according to the Soxhlet method described by [18].

REAGENT PREPARATION

In the reagent preparation, 250ml of 100µM of DPPH (2,2-Diphenyl -1-picrylhydrazyl) was prepared. Also 100ml of 1000µg/ml BHA was also prepared for the running of the analysis.

ANALYSIS

The DPPH scavenging activity of the extract was determined by the method as described [19].

RESULTS

From the experiment carried out using standard methods, it was observed that in the different test tubes from 0 to 5 having different concentrations of the sample, the higher the concentration the darker the green colour of the sample. The first test tube which the blank with 0µg/ml of the plant extract, the purple colour of DPPH was retained after 30 minutes and in the remaining 5 test tubes having the plant extract at different concentrations, the purple colour of DPPH disappeared after 30 minutes. Also for the standard, the purple colour of DPPH was retained in the test tube with 0µg/ml of BHA which is the blank but in the remaining five (5) test tubes containing different concentration of BHA the purple colour of DPPH disappeared after 30 minutes in the dark, showing that the plant extract and BHA has a reducing ability on DPPH which is a standard radical.

Table 1: showing the absorbance of the sample at different concentrations of *Justicia carnea* leaves

µg/ml	Sample	Methanol	DPPH	517nm
0	0	1	1	1.310
200	0.2	0.8	1	0.649
400	0.4	0.6	1	0.194
600	0.6	0.4	1	0.183
800	0.8	0.2	1	0.175
1000	1	0	1	0.154

Table 2: Showing absorbance of BHA at Different Concentrations

Concentration (µg/ml)	Volume of BHA	Volume of methanol	Volume of DPPH	Absorbance (517nm)
0	0	1	1	1.310
200	0.2	0.8	1	0.053
400	0.4	0.6	1	0.054
600	0.6	0.4	1	0.046
800	0.8	0.2	1	0.044
1000	1	0	1	0.073

Table 3: Showing the Percentage Radical Scavenging Activity of BHA and Sample at Different Concentrations

Concentration (µg/ml)	Percentage RSA of *J. carnea* leaves	Percentage RSA of the Standard (BHA)
0	0	0
200	50.46	95.95
400	85.19	96.11
600	86.64	96.49
800	86.04	96.72
1000	88.24	96.72

GRAPH OF % RSA AGAINST CONCENTRATION

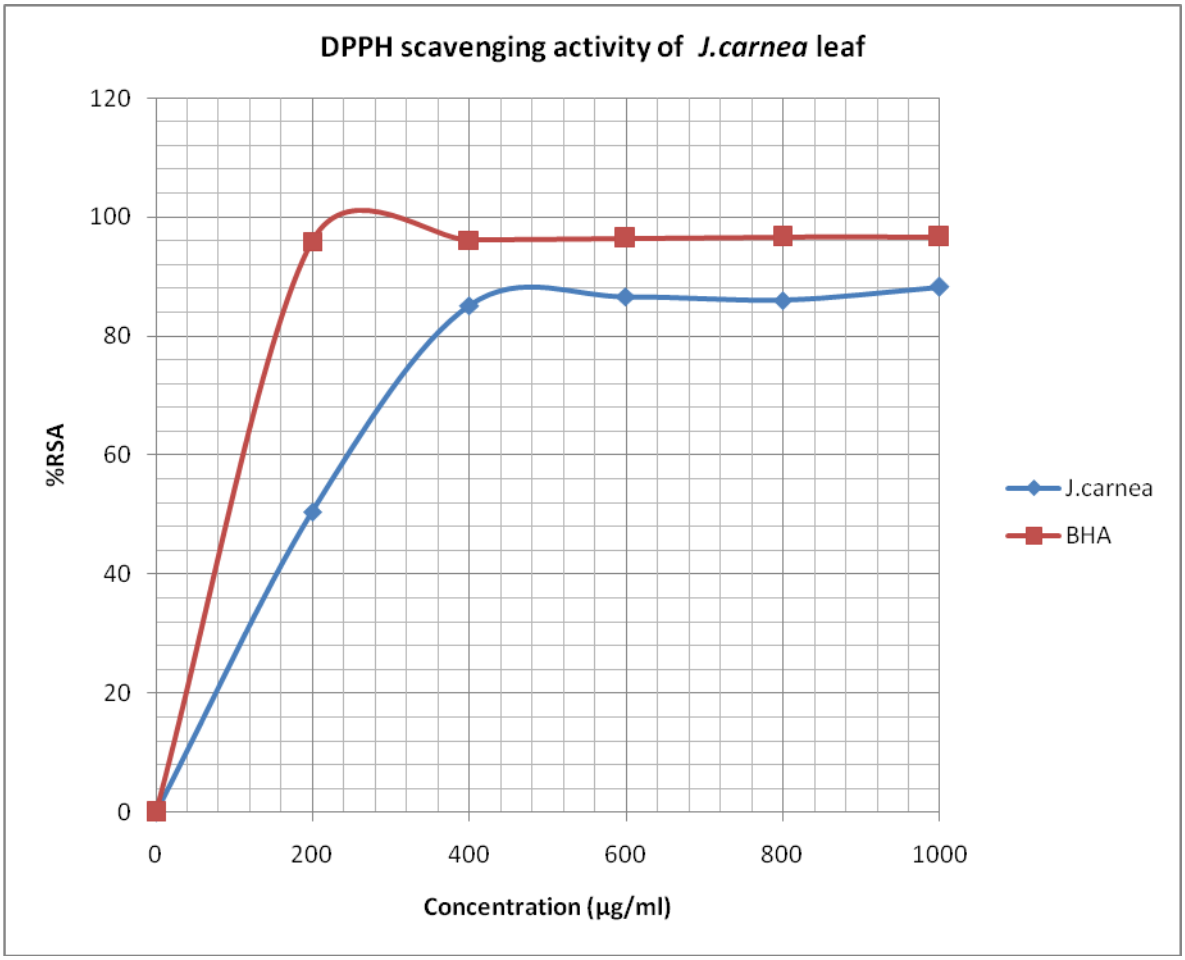

Fig 1 Graph of DPPH scavenging activity of *Justiciacarnea* leaf (Blue curve) compared with a standard antioxidant, Butylated hydroxyanisole (Red curve).

EC_{50} for *J.carnea* is 544.87µg/ml

EC_{50} for BHA is 739.78 µg/ml

DISCUSSION

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. *Justicia carnea* (*Justicia Purpurea L.)* has biological and chemical information [1].*Justiciacarnea* contains lignans which one the major components of the active extracts of the species of *Justicia*, exhibiting important pharmacological properties. From the work carried out, DPPH which is a known radical was used to determine the scavenging activity or the electron donating ability of the leaves of *Justiciacarnea*. From table 2 the concentration of the sample increased from 0– 1000µg/ml, the absorbance decreased gradually showing that the higher the concentration the lower the absorbance.This shows that the sample extract has chemical components with the ability to reduce DPPH by donating an electron which was indicated by the disappearance of the violet colour of DPPH on addition to the sample extract. From table 3 Showing the percentage radical scavenging activity of BHA and sample at different concentrations. RSA increased gradually as concentration increased from 0 – 1000µg/ml showing that the leave extract of*Justicia carnea* has ability to reduce free radical by donating an electron. Also from the table the value for % RSA of *Justicia carnea*leaves extract at 1000µg/ml and BHA which is the standard is very close, showing that the methanol extract of *Justicia carnea* leaves have a high radical scavenging activity because only at 400µg/ml, the

%RSA of the leaves extract was already 85.19.From the graph of DPPH Scavenging activity of *Justicia carnea*leaf compared with a standard antioxidant, butylated hydroxyanisole, the curves are very close to each other showing that they have close % RSA. From the graph also, the EC50 for *Justicia carnea* is 544.87µg/ml and EC50 for BHA is 739.78µg/ml. the lower the EC_{50} the higher the activity. This shows that *Justicia carnea*with 544.87µg/ml has more activity when compared with BHA which is the standard with EC50 739.78.

CONCLUSION

From the study carried out it can be concluded that *Justicia carnea*leaf have compounds with thepotential to scavenge free radicals by donating electrons that stabilizes free radicals. These free radicals cause oxidative stress. Therefore, *Justicia carnea* leaves are rich sources of antioxidants.

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