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DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Radical Scavenging Activity of Some Ethnomedicinal Plants in Nigeria

C.A. Anosike, N.E. Ogbodo, A.L. Ezugwu, R.I. Uroko, C.C. Ani and O. Abonyi

Department of Biochemistry, University of Nigeria Nsukka, Enugu State Nigeria

Abstract: The study evaluated the free radical scavenging activity of some ethnomedicinal plants in Nigeria using 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) as a model for determining the antioxidant activity of the plants. A total of twelve plants were used while ascorbic acid served as a standard. They were *Amaranthus spinosus, Telfairia occidentalis, Ocimum gratissimum*, *Solanum nigrum*(leaves); *Allium sativum, Allium cepa,, Zingiber officinale* (spices); *Lycopersicum esculentum, Solanum aethiopicum*, *Xylopia aethiopica* (fruits) and *Hibiscus sabdariffa* and *Garcinia kola*. The test samples and standard (ascorbic acid) showed a significant anti-radical power (ARP) which is a measure of the antioxidant activity and is the inverse of EC_{50} (effective concentration that causes fifty percent inhibition of $DPPH^{\cdot}$). The result of the antioxidant activity showed the following trend: *Telfairia occidentalis* and *Allium sativum* >*Amaranthus spinosus* >*Xylopia aethiopica* >*Allium cepa*, *Solanum nigrum* >*Ocimum gratissimum*, *Lycopersicum esculentum* >*Zingiber officinale*, *Hibiscus sabdariffa and Garcinia kola*> *Solanum aethiopicum*>Ascorbic acid.

Key words: DPPH Radical · Free Radical Scavenging Activity · Antioxidant Activity · Anti-Radical Power · Ethnomedicinal Plants

INTRODUCTION

Medicinal plants have various effects on living systems which include sedative, analgesic, antipyretic, cardioprotective, antibacterial, antiviral and antiprotozoal among others [1]. The specific constituents which impact medicinal values on the plants can be derived from whole or parts of the plant such as stems, leaves, fruits, flowers, seeds and roots [2]. The growing public interest and awareness in herbal medicine have led the pharmaceutical industry and biomedical researchers to give more attention on medicinal plants [3]. The current relative shift to the use of herbal preparations in many countries may be ascribed to the presumed effectiveness, relative low cost, presumed less side effects and low toxicity, with respect to orthodox drugs, even though the biologically active constituents, may often be unknown [4]. The major problem in the preparations and acceptance of herbal therapy in developing countries, are adulteration and misidentification. The situation is aggravated by the absence of quality control system. Some that are involved in herbal preparations, still employ organoleptic testing like sight, smell, taste and touch in identifying a plant. Consequently the practice is considered unscientific [5]. Moreover, non-availability of the plants (some are seasonal), nomenclatural confusion, morphological similarity, careless collection, lack of knowledge of authenticity of the plants and other unknown reasons are militating factors in the usage of herbal remedies. Other problems, with no less significance, are difficulty associated with the method of usage of some medicinal plants which differs from one area to another [6] and the inadequate information available on the phytochemistry, pharmacology and toxicity of some of these plants [7, 8].

In spite of all these limitations, many of these medicinal plants are good sources of antioxidants which are substances that delay oxidation process, inhibiting the polymerization chain reaction initiated by free radicals and other subsequent reactions (propagation reactions) [9]. These antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals [10]. Oxidative damages caused by free radicals are related to various diseases [11]. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione [12 , 13]. Free radicals have been implicated in the etiology of several major human ailments including

Corresponding Author: O. Abonyi, Department of Biochemistry, University of Nigeria Nsukka, Nsukka Enugu State Nigeria.

cancer, cardiovascular diseases, neural disorders, diabetes, atherosclerosis, myocardial infarction and also in the ageing process [14, 15]. A diet rich in fruit and vegetables has been associated with a reduced risk of developing these chronic diseases [16, 17] and age-related neurodegenerative diseases. Hence, the need to evaluate the antiradical potentials of some ethnomedicinal plants using DPPH radical scavenging assay so as to advance knowledge in their usage for the purpose of ameliorating these debilitating disease conditions caused by free radicals.

MATERIALS AND METHODS

Plant Materials: Twelve (12) plant materials were obtained from Enugu and Nsukka markets both in Enugu state Nigeria. The plant materials include *Telfairia occidentalis*, *Allium sativum, Amaranthus spinosus, Xylopia aethiopica, Allium cepa*, *Solanum nigrum, Ocimum gratissimum*, *Lycopersicum esculentum, Zingiber officinale*, *Hibiscus sabdariffa, Garcinia kola*, *Solanum aethiopicum.* They were identified by Mr Alfred Ozioko of Biodiversity and Conservative Programme (BDCP) Nsukka.

Chemicals: The chemicals used were of analytical grade and are products of Sigma Aldrich Germany.

Extraction: The plant materials were collected and sun dried for three weeks. They were pulverized using a mechanical grinder. This was followed by 24-hour maceration in methanol, double filtration using filter cloth and Whatmann filter paper respectively and concentration using rotary evaporation.

Quantitative DPPH Radical Scavenging Assay: The scavenging activity on $DPPH^{\cdot}$ free radicals by the extract was done using the method of Gyamfi *et al.* [13] with slight modifications. One milliliter (1ml) of the extracts at different concentrations (20-100µg/ml) was obtained by diluting 10-fold in 80% methanol and allowed to stand at room temperature in the dark for 30 minutes. The negative control was 0.063 mM $DPPH^{\cdot}$ in methanol. L-ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixtures was read using Jenway 6405 UV/VIS spectrophotometer (Beckman/Instruments, Inc., Huston Texas) at 515 nm and methanol was used to zero the instrument. DPPH radical scavenging activity was calculated using the equation:

$$\% \text{ scavenging activity} = \frac{\text{Ao-As}}{\text{Ao}} \text{x} 100$$

where Ao = absorbance of negative control, As = absorbance of test sample, The EC_{50} value represented the concentration of the sample leading to 50% reduction of the initial $DPPH^{\cdot}$ This was calculated using a plot of % inhibition against different concentrations of the extract.

RESULTS AND DISCUSSION

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay uses $DPPH^{\cdot}$ Which is a stable free radical with a maximum absorption at about 515nm [18]. The concentration in of an antioxidant that decreases the initial DPPH concentration by fifty percent (50%) is the EC_{50}. The drawbacks of this assay are mainly due to the reactivity of the DPPH radical, which may react slowly or not react at all with some antioxidants, possible interference with compounds that present UV-visible absorption maxima around 515nm, leading to underestimations [19]. However, it is still widely used because of its repeatability [20].

The findings from this study indicated little or no change in the percentage inhibition of the DPPH radical by the test samples with progressive decrease in concentration (Table 1). This may be due to the fact that the various concentrations used are within the steady state region [21]. *Telfairia occidentalis* showed the highest antioxidant activity while *Ocimum gratissimum* showed the least antioxidant activity (Figs. 1a-d; Table 2) among the leaves class. The spices class revealed that *Allium sativum* had the highest while *Zingiber officinale* had the lowest antioxidant activity. The fruit class indicated that *Xylopia aethiopica* showed the highest while *Solanum aethiopicum* had the least antioxidant activity. It was, also observed that *Hibiscus sabdariffa* had higher antioxidant activity compared to *Garcinia kola*. The high antioxidant activity observed in some of these test samples might be as a result of the presence of polyphenols, monoterpenes, an essential oil and α-tocopherol, among others. *Telfairia occidentalis* was reported to have flavonoids and phenols while *Allium cepa* has flavonoids [22, 23], *Ocimum gratissimum* and *Xylopia aethiopica* have essential oils [24]. *Hibiscus sabdariffa*, *Garcinia kola* and *Solanum aethiopicum* were reported to have flavonoids and ascorbic acid [25, 26]. These bioactive constituents present in these plant materials have strong antioxidant properties.

Table 1: Effect of Concentration on Percentage Inhibition of DPPH radical in Methanol by Extracts of the Test Samples

Test samples	Concentration (µg/ml)	%inhibition
Amaranthus spinosus (green)	100	99.00±0.00
	50	98.00±0.00
	33	97.33±0.58
	25	98.00±1.73
	20	98.33±2.08
Telfairia occidentalis (ugu)	100	98.67±0.33
	50	98.67±0.33
	33	98.33±0.33
	25	99.33±0.33
	20	99.00±0.00
Ocimum gratissimum (scent leaf)	100	94.33±2.19
	50	96.67±0.33
	33	97.67±0.88
	25	97.67±0.67
	20	98.33±0.33
Xylopiaaethiopicum (uda)	100	96.33±0.33
	50	97.33±1.20
	33	96.67±1.67
	25	97.33±2.19
	20	97.00±2.08
Allium sativum (garlic)	100	99.67±0.33
	50	99.67±0.33
	33	100.00±0.00
	25	98.67±0.33
	20	98.33±0.67
Allium cepa (onion)	100	95.67±2.19
	50	96.00±1.53
	33	97.33±1.67
	25	97.33±1.67
	20	95.00±1.53
Lycospersicum esculentum (tomato)	100	95.33±3.84
	50	94.33±3.71
	33	95.67±0.88
	25	96.33±0.88
	20	95.33±0.58
Solanum nigrum (leaf)	100	97.33±0.33
	50	97.00±0.58
	33	95.67±0.67
	25	96.00±0.58
	20	95.00±0.58
Zingiber officinale (ginger)	100	95.33±0.33
	50	96.00±0.00
	33	94.33±0.33
	25	95.33±0.33
	20	91.00±0.58
Hibiscus sabdariffa (zobo)	100	96.67±0.88
	50	92.33±0.33
	33	91.33±0.33
	25	91.67±0.33
	20	92.00±1.15
Garcinia kola (bitter cola)	100	91.33±0.88
	50	91.67±0.33
	33	91.00±0.58
	25	91.00±0.58
	20	92.00±0.00
Solanum aethiopicum (garden egg)	100	91.67±0.33
	50	90.67±0.67
	33	91.33±0.67
	25	90.33±0.33
	20	90.67±0.88

Table 2: The EC_{50} of ascorbic acid (standard) and test samples

	Samples EC_{50} (µg/ml)
Amaranthus spinosus (green)	8.28
Telfairia occidentalis (ugu)	1.13
Ascorbic acid	673.47
Ocimum gratissimum (scent leaf)	30.63
Xylopia aethiopicum (uda)	15.57
Allium sativum (garlic)	1.13
Allium cepa (onion)	25.51
Zingiber officinale (ginger)	57.16
Solanum nigrum (leaf)	25.51
Lycospersicum esculentum (tomato)	30.63
Hibiscus sabdariffa (zobo)	54.44
Garcinia kola (bitter cola)	57.16
Solanum aethiopicum (garden egg)	62.73

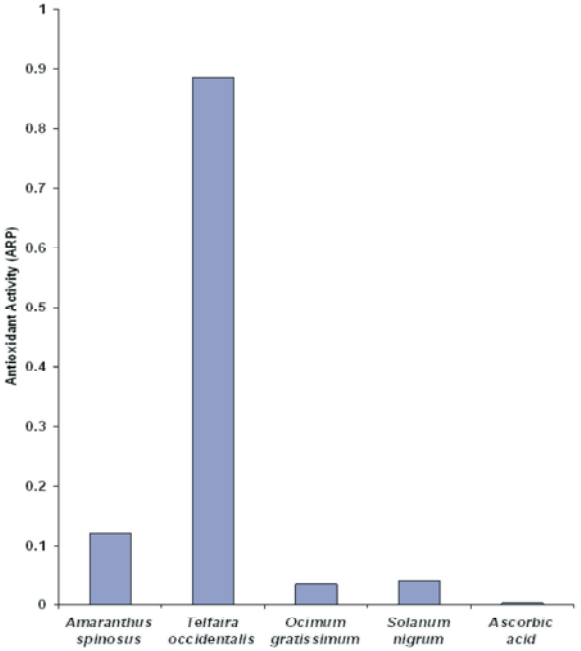

Fig. 1a: Antioxidant activity of leaves samples

These plants could, therefore, be employed in tackling health problems associated with free radicals. Those that have high antioxidant activity could be integrated more into diets, especially when high fever is suspected, among infants, to cushion the deadly effect of free radicals seen in pyrexia and which is one of the leading causes of deaths among infants. *Zingiber officinale* and the likes (with lower antioxidant activity) are encouraged while taking some drugs like quinone and beta-lapachome (an antiplatyhelminthes) used in treating trypanosomias is whose mode of action is by generation of reactive free radicals. This is to enable the drug to act maximally. Those with high antioxidant activity can, also be used as food additives since crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in

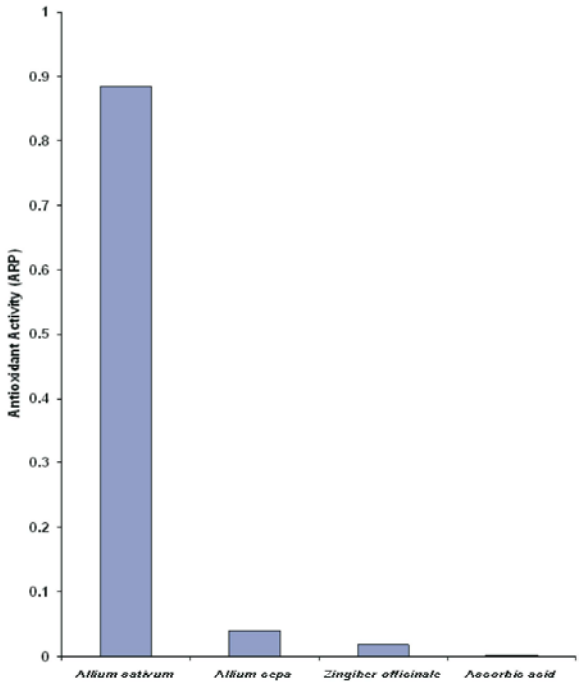

Fig. 1b: Antioxidant activity of spices samples

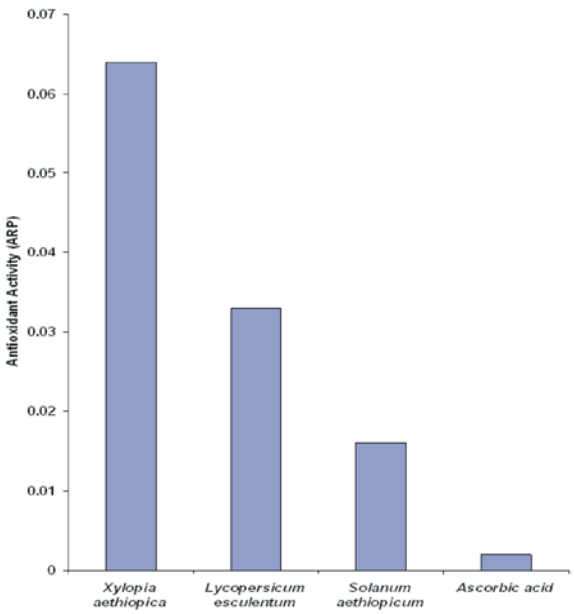

Fig. 1c: Antioxidant activity of fruit samples

phenolics are gaining increased interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [27]. The ascorbic acid that was found to have the lowest antioxidant activity might be due to the presence of only two abstractable protons in it which is small compared to many protons in polyphenols [28].

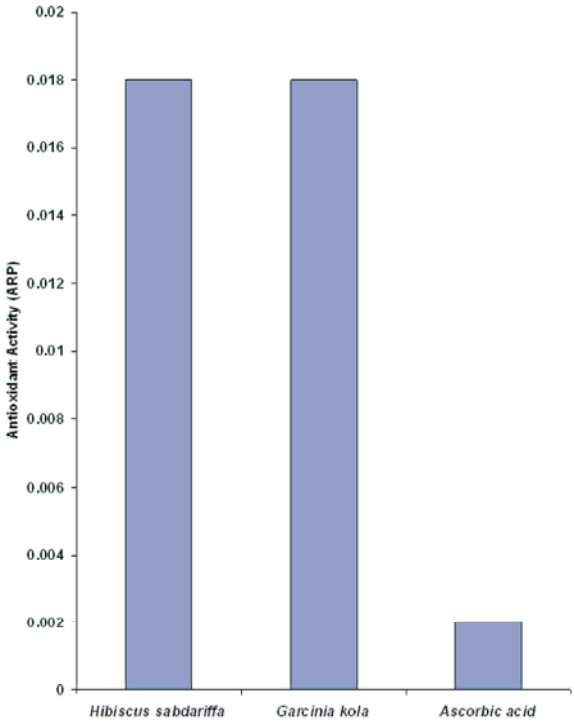

Fig. 1d: Antioxidant activity of other samples

In addition, these plants could serve as good sources of phenolic compounds and related compounds and therefore could be used as good sources of drugs whose mode of action is by chelating free radicals just like the phenolate ion from phenols. Besides chelation, polyphenols donate hydrogen atom (H) to free radicals thereby quenching their effects such as free radical propagation which is deleterious to membrane lipids. If this propagation were not halted, it could lead to membrane instability which would ultimately lead to malfunctioning of the cells; since cell membrane integrity is paramount to cell integrity, cell function and the entire well being of the organism [29].

CONCLUSION

Numerous studies have demonstrated that antioxidant activity measured depends substantially on the test system used [30] and it is recommended to base any conclusions of antioxidant activity on at least two different systems [31]. It is, therefore important to employ other systems such as FRAP (Ferric Reducing/ Antioxidant Power) and NBT (nitro blue tetrazolium) methods to further give credence to these findings. Also, *in vivo* study would help immensely in projecting the antioxidant activity of these medicinal plants.

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