Available at http://www.newresearch.co.in/
World Research Journal of
PHARMA TECHNOLOGY
ISSN: 2454-5546
Research Article

IN VITRO EVALUATION OF FREE RADICAL SCAVENGING ABILITY OF ROOT BARK EXTRACTS OF MASSULARIA ACUMINATA SPECIES

Uduak S. Ukekpe[1, 2]*, Eno O. Ekanem[1], Harami M. Adamu
[1] *Chemistry programme, Abubakar Tafawa Balewa University, Bauchi, Nigeria.*
[2] *Department of Science Laboratory Technology, Federal Polytechnic, Damaturu, Nigeria.*

Corresponding Author
Uduak S. Ukekpe

Chemistry programme, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

Department of Science Laboratory Technology, Federal Polytechnic, Damaturu, Nigeria.

E-mail: uukekpe@yahoo.com

Recieved on: Feb 25, 2016

Accepted on: March 22, 2016

Published on: April 15, 2016

ABSTRACT
The root bark of Massularia acuminata has been used for medicinal purpose in Akwa Ibom State, Nigeria. The plant part has been established to be rich in some phytochemicals. This work evaluates the free radical scavenging ability and hence antioxidant property of ethyl acetate, acetone, ethanol and methanol extracts of the plant part by the DPPH radical scavenging method. Ethyl acetate extract exhibited the least radical scavenging ability of 49.71±0.17 % at 100µg/ml concentration. But methanol, ethanol and acetone extracts show appreciable scavenging ability of 67.47±0.30, 65.01±0.15 and 63.64±0.17 % respectively, values which are comparable with that of the common antioxidant, ascorbic acid. Therefore the three extracts can be said to possess reasonable antioxidant property. The variation in the free radical scavenging ability of the extracts shows that extracting solvents play a role in the types of phytochemicals extracted and hence antioxidant property. Based on the outcome of this work, the plant part can be said to possess antioxidant phytochemicals which can be used to manage oxidative stress.

Keywords: *Free Radicals, Antioxidants, Extracts, Phytochemicals Scavenging, Root bark.*

INTRODUCTION

Free radicals constitute one of the by-products of metabolism in the body (Davies, 1995). They are known to be beneficial and damaging species in the body (Gaikwad *et al*., 2011) They are signalling molecules in the body at the right concentration (Sen and Chakraborty, 2011, Held, 2012), and, as they are oxidants, excess production in the body leads to oxidative stress, damage to DNA, proteins and lipids leading to pathological manifestation in form of degenerative diseases such as cancer, Alzheimer's disease, diabetes, Parkinson's disease, inflammatory condition and others (Sen *et al*., 2010, Gaikwad *et al*., 2011).

The deleterious oxidative activity of free radicals in the body is usually halted by antioxidants. These antioxidants are

Available at http://www.newresearch.co.in/
World Research Journal of
PHARMA TECHNOLOGY
ISSN: 2454-5546
Research Article

compounds that prevent or act against oxidative process of free radicals (Venkatesh and Sood, 2011). Antioxidants therefore have the ability to protect organisms from damage caused by free radical induced oxidative stress (Guyal *et al*., 2010). Antioxidants act or produce their protective role against free radicals by scavenging or quenching them, inhibiting their formation, chelating transition metal ions that catalyze their formation, terminating or breaking auto-oxidative chain reaction, reducing localized O_2 concentration or by any combination of the aforementioned (Gupta and Sharma, 2006, Sen and Chakraborty, 2011, Brewer, 2011).

There are synthetic and natural occurring compounds used as antioxidants. However, toxicity of synthetic antioxidants has been observed (Rajan *et al*., 2011). Natural occurring antioxidants have therefore drawn much attention because of their relative safety and potential nutritional and therapeutic effects (Mandal *et al*., 2009). These natural antioxidants are of plant origin and are phytochemicals. Many classes of phytochemicals have been found to possess antioxidant property. Sen *et al*. (2010) have listed these classes to include flavonoids, triterpenoid saponins, Phenolic acids, flavonols, flavones, coumarins, tannins and alkaloids. These compounds have been reported to be good singlet and triplet oxygen quenchers, radical scavengers and peroxide decomposers (Mandal *et al*., 2009).

The phytochemical study of the plant *Massularia acuminata*, a common chewing stick in Southern Nigeria have shown the stem to have some of the listed antioxidant phytochemicals as it phytoconstituents (Yakubu *et al*., 2008, Akande and Ajao, 2011, Maloueki *et al*., 2015). The root bark of the plant has also been reported to have these antioxidant phytochemicals (Ukekpe *e*t *al*., 2015). In this work, the free radical scavenging ability, hence antioxidant property of ethyl acetate, acetone, ethanol and methanol extracts of the root bark of *Massularia acuminata* has been evaluated. The 1,1-diphenyl-2-picrulhydrazyl (DPPH) radical scavenging method is employed. In the method, the DPPH serves as the oxidizing radical to be reduced as well as the colour indicator for the reaction.

MATERIALS AND METHODS

Plant collection and Sample Preparation.

The plant, *Massularia acuminata* was collected from Ika local Government Area

Available at http://www.newresearch.co.in/
World Research Journal of
PHARMA TECHNOLOGY
ISSN: 2454-5546
Research Article

of Akwa Ibom State, Nigeria and identified at the botanical laboratory of University of Uyo, Nigeria.

Fresh roots of the plant were collected, washed with water, the bark peeled- off, air dried and ground to fine powder.

Extraction

The powdered plant material was extracted with hexane, chloroform, ethyl acetate, acetone, ethanol and methanol sequentially in that order by Soxhlet method. The ethyl acetate, acetone, ethanol and methanol extracts were each concentrated using rotary evaporator, dried in moist free environment and used for the antioxidant assay.

Free Radical Scavenging Assay

The antioxidant activity of each of the ethyl acetate, acetone, ethanol and methanol extracts was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method reported by Hemalatha *et al*. (2010), Khalaf *et al*. (2008) and Walia *et al*. (2010). The DPPH solution, 0.1mM, was prepared in 95% methanol and varying concentrations (20, 40, 60, 80 and 100µg/ml) of each of the extract solutions were also prepared in 95% methanol. $3cm^3$ of each extract solution was added to $1cm^3$ of the DPPH solution and the absorbance measured at 517nm after 30 minutes using JENWAY 6305 spetrophotometer. The absorbance of the blank ($3cm^3$ 95% methanol added to $1cm^3$ DPPH solution) was also measured.

The radical scavenging activity of each of the extracts' solution was expressed in percentage of the ratio of lowering of the absorbance of DPPH by the extract solution relative to the absorbance of DPPH $\{(A_{DPPH} - A_{Sample})/ A_{DPPH}\}$ x 100%, where A is the absorbance. The IC_{50} was obtained by regression and the method of Rajan *et al*. (2011)

Available at http://www.newresearch.co.in/
World Research Journal of
PHARMA TECHNOLOGY
ISSN: 2454-5546
Research Article

Results

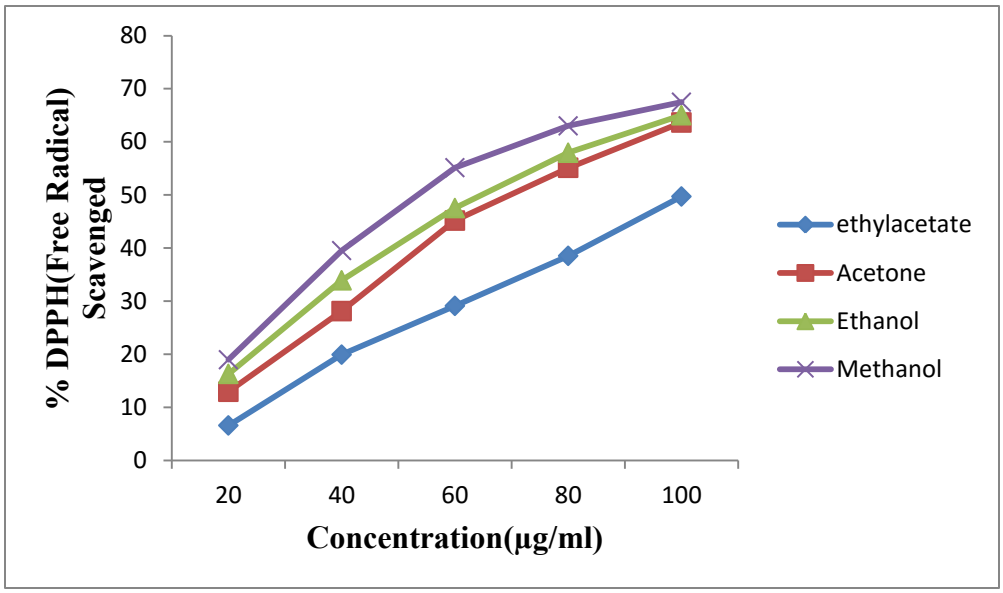

Fig. 1: Free radical antioxidant activities of ethyl acetate, acetone, ethanol and methanol extracts of *Massularia acuminate*.

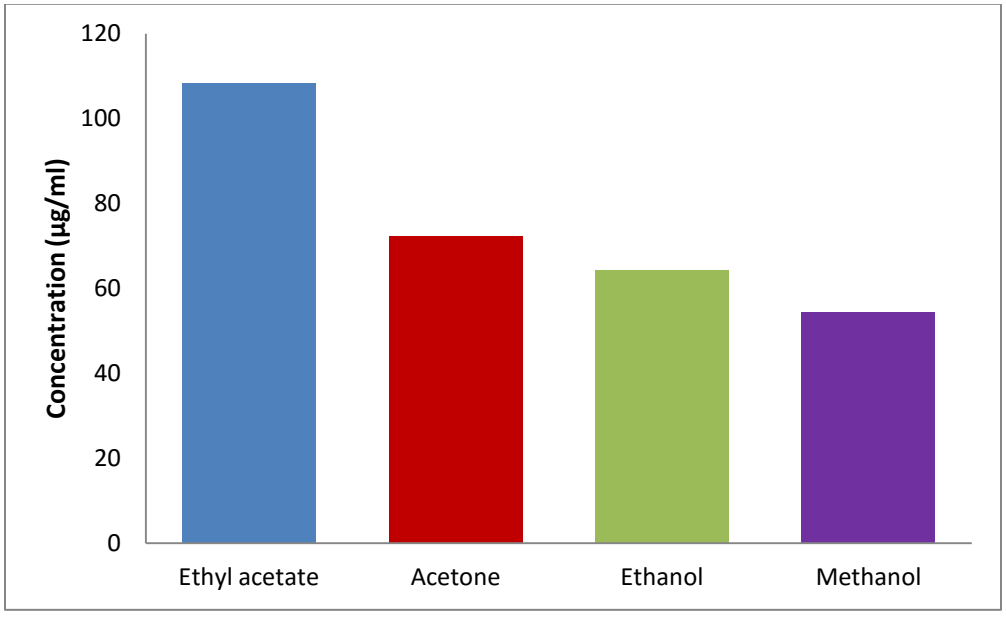

Fig. 2: IC_{50} of Ethyl acetate, Acetone, Ethanol and Methanol Extracts of *Massularia acuminate*.

Available at http://www.newresearch.co.in/
World Research Journal of
PHARMA TECHNOLOGY
ISSN: 2454-5546
Research Article

DISCUSSION

The free radical scavenging ability study of the ethyl acetate, acetone, ethanol and methanol extracts of the root bark of *Massularia acuminata* **(Figure 1)** show all the extracts to exhibit free radical scavenging ability though at varied degrees. The ethyl acetate extract exhibited the least free radical scavenging ability (49.71±0.17 % at 100µg/ml). This is as expected, based on the phytochemical constituents of the extract as reported by Ukekpe *et al*. (2015). In the work, it is reported that the extract contains only saponins and terpenoids as the phytochemicals with antioxidant property. This is as oppose to the presence of other antioxidant phytochemicals, flavonoids, tannins and phlobatanins along with saponins and terpenoids in the acetone, ethanol and methanol extracts as reported in the same work. Correspondingly, the results show methanol extract to exhibit the highest free radical scavenging ability followed by ethanol and acetone in that order at all concentrations **(Figure 1)**.

At 100 µg/ml methanol extract showed 67.47 ± 0.30% scavenging ability, ethanol, 65.01±0.15% and acetone 63.64±0.17%, all higher than the 49.71±0.17% of ethyl acetate. This shows that extracting solvent has effect on free radical scavenging ability of extracts. This is so because the solvents may not extract the same range of phytochemicals and in the same quantity. Polarity of the solvent and the chemical nature of individual phytochemicals are factors influencing the phytochemical profile and quantity that the solvent can extract. The work of Ukekpe *et al*., (2015) has also shown this as it was observed that methanol extracted more phytochemicals from the plant part than ethanol and acetone with only a few groups of phytochemicals of the plant part being soluble in ethyl acetate. This observation is in consonant with the

findings of Sultana, *et al*., (2009) on the effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. In the study, it was found that extracts of more polar solvents have higher free radical scavenging activity than that of the less polar solvents. They also stated that the resulting antioxidant activities of plant materials are strongly dependent on the nature of extracting solvent due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent.

Comparatively, the plant part can be said to contain reasonable amount of antioxidants as exhibited by the free radical scavenging ability of acetone, ethanol and methanol extracts as the values are comparable to that of the common standard, ascorbic acid, reported by Khalaf *et al*. (2007). This is also corroborated by the IC_{50} of the extracts as acetone, ethanol and methanol extracts show IC_{50} of 72.10, 64.12 and 54.21 µg/ml respectively **(Figure 2)**, compared with 55.89 µg/ml reported for ascorbic acid by Saha *et al*. (2008). However, ethyl acetae extract IC_{50} tof 108.21 µg/ml is an indication of its poor antioxidant property. Methanol, ethanol and acetone extracts have therefore shown the potential that they can be used to manage oxidative stress.

CONCLUSION

The outcome of this investigation has shown that the root bark of *Massularia acuminata* contains phytochemicals that are antioxidant in nature and are capable of scavenging free radicals reasonably. This has shown the plant part to possess medicinal value as the extracts can be used to manage oxidative stress based on the free radical scavenging potential. It has been established that there is a relationship between extracting solvent and the antioxidant property of extracts as it affects the composition of phytochemicals

extracted. Methanol has been observed to be a good solvent for extracting antioxidant phytochemicals from the root bark of *Massularia acuminata* as revealed by the free radical scavenging power of the methanol extract.

References.

Akande, T.A. and Ajao, A.T. (2011). Chemotherapeutic Values of Four Nigerian Chewing Sticks on Bacteria Isolates of Dental Infection. *Global Journal of Science and Frontier Research*. **11**(8): 91 – 95.

Brewer, M. S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of Action and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety.* **10**. 221 – 247.

Davies, K. J. (1995). Oxidative Stress: The Paradox of Aerobic Life. *Biochemical Society Symposia.* **61**. 1 -31.

Gaikwad, S. A., Kamble, G. S., Devare, S., Deshpande, N. R. and Salvekar, J. P. (2011). *In vitro* Evaluation of Free Radical Scavenging Potential of *Cassia auriculata* L. *Journal of Chemical and Pharmaceutical Research*. **3**(4). 766 – 772.

Gupta, V. K. and Sharma, S. K. (2006). Plants and Natural Antioxidants. *Natural Product Radiance.* **5**(4). 326-334.

Goyal, A. K., Middha, S. K. and Sen, A. (2010). Evaluation of the DPPH Radical Scavenging Activity, Total Phenols and Antioxidant Activities in Indian Wild *Bambusa vulgaris* "vittata" Methanolic Leaf Extract. *Journal of Natural Pharmaceuticals*. **1**(1). 40-45.

Held, P. (2012). An Introduction of Reactive Oxygen Species: Measurement of ROS in Cells. *Biotek Application Guide.* Retrieved from www.biotek.com/assets/tech_resources/ROS%20%Application.pdf on 20/10/2014.

Hemalatha, S., Lalitha, P. and Arulpriya, P. (2010) Antioxidant Activities of the Extracts of the Aerial Roots of *Pothoscura* (Linden ex Andre). *Der Pharma Cehmica*. **2** (6). 84 – 89.

Mandal, S., Yadav, S., Yadar, S. and Nema, R. K. (2009). Antioxidants: A Review. *Journal of Chemical and Pharmaceutical Research.* **1(1)**. 102 – 102.

Khalaf, N.A., Shakya, A.K., AL-Othman, A., El-Agbar, Z. and Farah, H. (2008). Antioxidant Activity of some Common Plants. *Turk J. Biol.* **32**: 51-55.

Maloueki, U. Kunyima, K. P., Mbomba, I., D., Dani, N., A.,Lukuka, K. A., Lami, N. J. *et al*. (2015). Antioxidant and Antiplasmodial Activities of

ISSN: 2454-5546
Research Article

Extracts of *Massularia acuminata* (Rubiaceae). *Phytoth*rapie *Vétérinaire*. 1-7.

Rajan, S., Gokila, M., Jency, P., Brindha, P. And Sujatha, R. K. (2011) Antioxidant and Phytochemical Properties of *Aegle marmelos* Fruit pulp. *International Journal of Current Pharmaceutical Research*. **3**(2**)**. 65-70.

Saha, M. R., Hasan, S. M. R., Akter, R., Hossein, M. M., Alam, M. S. and Mazumder. M. E. H. (2008). In *Vitro* Free Radical Scavenging Activity of Methanol Extract of the Leaves of *Mimusop elengilinn*. *Ba*ngladesh *Journal of Veterinary Medicine*. **6**(2): 197 – 202.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S. R. and De, B. (2010). Free Radicals, Antioxidants, Diseases and Phytomedicines: Current Status and Further Prospect. *International Journal of Pharmaceutical Sciences Review and Research.* **3(1)**. 91- 100.

Sen, S. and Chakraborty, R. (2011). *The Role of Antioxidants in Human Health.* In: Oxidative Stress; Diagnostics, Prevention and Therapy. Ed. Andreescu, S. 2011 American Chemical Society Symposium Series. P1 – 37.

Sultana, B., Anwar, F. Ashraf, M. (2009) Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules*. **14**: 2167 – 2180.

Ukekpe, U. S., Adamu, H. M., Ekanem, E. O. and Saleh, A. A. (2015). Phytochemical Constituents and Antimicrobial Activities of the Root Bark Extracts of *Massularia acuminata spp*. *International Journal of Advance Research*. **3**(8): 557 – 565

Venkatesh, R. and Sood, D. (2011) *A Review of the Physiological Implications of Antioxidants in Food*. An Interactive Qualifying Project Report Submitted to Worcester Polytechnic Institute. Retrieved from http://www.wpi.edu/pubs/E-project/Available/E-Project-011411-11141/ unrestricted/rvenkatesh=disha-1qp.pdt on 20/11/2013.

Walia, H., Singh, R., Kumar, S. and Arora, S. (2010) Effect of Fractionation on Antiradical efficacy of Ethyl Acetate Extract of *Terminalia chebula* Retz. *African Journal of Pharmacology.* **4** (5): 276 – 2851.

Yakubu, M. T., Akanji, M, A., Oladiji, A. T. Adesokan, A. A. (2008). Androgenic Potentials of Aqueous Extracts of *Massularia acuminata* (G. Don) Bullock ex. Hoyle. Stem in Male Wistar Rats. *Journals of Ethnopharmacology*.**118**: 508-513.