



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

***In vitro* EVALUATION OF FREE RADICAL SCAVENGING ABILITY OF ROOT
BARK EXTRACTS OF *Massularia acuminata* spp.**

*Uduak S. Ukekpe^{1,2}, Eno O. Ekanem¹ and Harami M. Adamu.

1. Chemistry Programme, Abubakar Tafawa Balewa University, Bauchi, Nigeria.
2. Department of Science Laboratory Technology, Federal Polytechnic, Damaturu, Nigeria.

Manuscript Info**Manuscript History:**

Received: 18 January 2016
Final Accepted: 11 February 2016
Published Online: March 2016

Key words:

Free Radicals, Antioxidants,
Extracts, Phytochemicals,
Scavenging, Root bark.

***Corresponding Author**

Uduak S. Ukekpe.

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 free radical scavenging method. Ethyl acetate extract exhibited the least free radical scavenging ability of 49.71 ± 0.17 % at $100 \mu\text{g/ml}$ concentration. But methanol, ethanol and acetone extracts showed 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 standard, 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.

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Introduction:-

Free radicals constitute one of the by-product of metabolism in the body (Davies, 1995). They are known to be beneficial and damaging species in the body (Gaikwad et al., 2011) as they are signalling molecules in the body at the right concentration (Sen and Chakraborty, 2011, Held, 2012), and in excess 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 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 its 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 et 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-picrylhydrazyl (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 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.

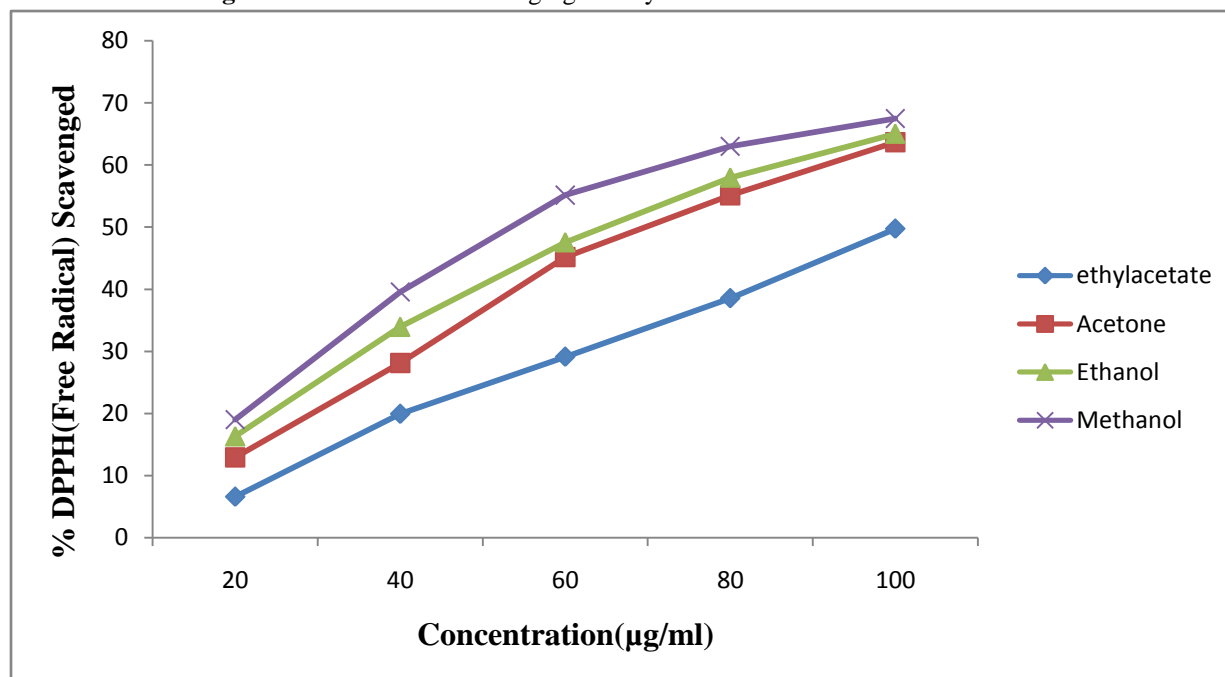
Free Radical Scavenging Assay:-

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. 3cm³ of each extract solution was added to 1cm³ of the DPPH solution and the absorbance measured at 517nm after 30 minutes using JENWAY 6305 spectrophotometer. The absorbance of the blank (3cm³ 95% methanol added to 1cm³ 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 $\{(A_{DPPH} - A_{Sample}) / A_{DPPH}\} \times 100\%$, where A is the absorbance. The IC₅₀ was obtained by regression and the method of Rajan et al. (2011)

Results:- Figure 1: Free Radical Scavenging Ability of Root Bark Extracts of *Massularia acuminata*.



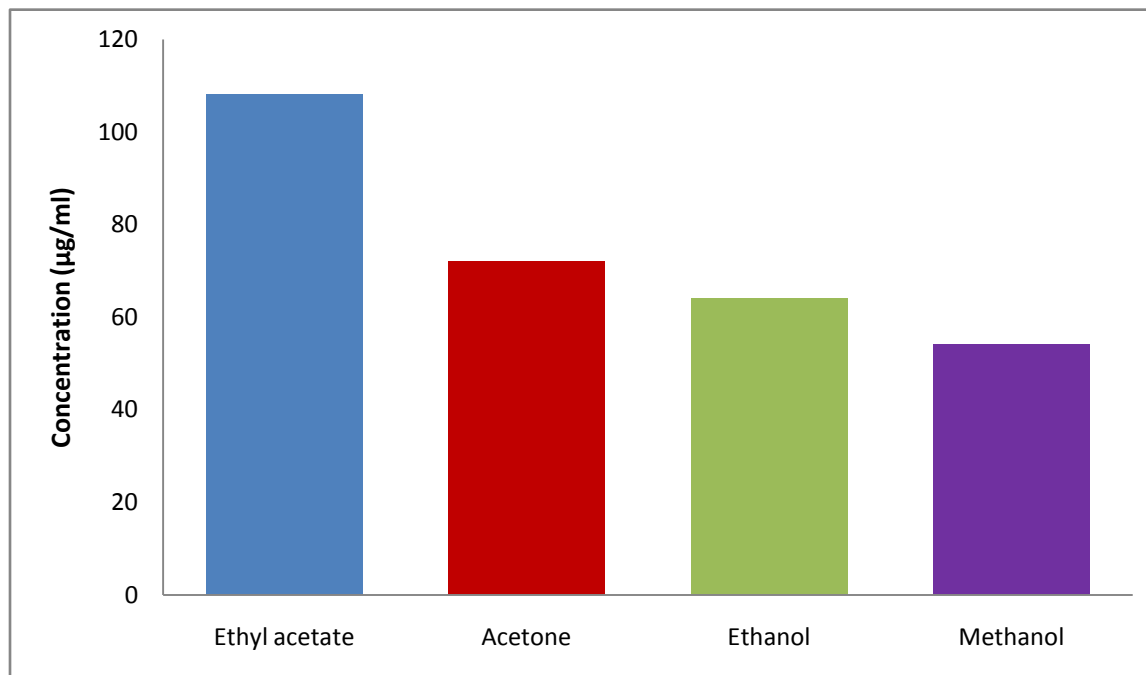


Figure 2: IC₅₀ of Root Bark Extracts *Massularia acuminata*.

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) showed all the extracts to exhibit free radical scavenging ability though at varied degrees. The ethyl acetate extract exhibited the least free radical scavenging ability ($49.71 \pm 0.17\%$ at $100 \mu\text{g/ml}$). 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 contained only saponins and terpenoids as the phytochemicals with antioxidant property. This is as opposed 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 showed methanol extract to exhibit the highest free radical scavenging ability followed by ethanol and acetone in that order at all concentrations (Figure 1).

At $100 \mu\text{g/ml}$ methanol extract showed $67.47 \pm 0.30\%$ scavenging ability, ethanol, $65.01 \pm 0.15\%$ and acetone $63.64 \pm 0.17\%$, all higher than the $49.71 \pm 0.17\%$ 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 with that of the common standard, ascorbic acid, reported by Khalaf et al. (2007). This is also corroborated by the IC₅₀ of the extracts as acetone, ethanol and methanol extracts show IC₅₀ of 72.10, 64.12 and 54.21 $\mu\text{g/ml}$ respectively (Figure 2), compared with 55.89 $\mu\text{g/ml}$ reported for ascorbic acid by Saha et al. (2008). However, ethyl acetate extract IC₅₀

of 108.21 μ g/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 extract.

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