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Evaluation of Antioxidant Potentials of *Alpina Muricata* Leaf

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Abstract
The present research was conducted to evaluate antioxidant potentials of Alpina muricata leaf extracts. Extracts were prepared from the leaf of the plant with aqueous, ethanolic and ethylacetate solvents system at 5.0% concentration. Free radical scavenging ability using 2,2-diphenyl-2-pricrylhydrazyl [DPPH] and metal chelation ability of the leaf extracts were assessed. Results revealed significantly ($P<0.05$) high antioxidant potential obtained from both aqueous and ethanolic extracts which was comparatively higher than that obtained from ethylacetate extract. Highest DPPH inhibition (97%) was exhibited by aqueous leaf extract of Alpina muricata plant, followed by (60%) ethanolic extract while the least DPPH inhibition (56%) was obtained in ethylacetate leaf extract. Besides, highest metal chelation potential (80%) was obtained from Alpina muricata leaf extract while the least metal chelation ability (50%) was demonstrated by ethylacetate leaf extract. The results from this investigation suggest that high radical scavenging potentials of Alpina muricata plant may be attributed to the hydrogen donating ability of the inherent phenolics.

Keywords: *Alpina muricata, 2,2-diphenyl-1-picryl hydrazyl, metal chelation, antioxidant potential.*

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I. Introduction

Several studies on phytomedicines have reported that phenolic compounds protect against oxidative stress [1]. Some of these medicinal plants have been investigated for their antioxidant properties and used for the treatment of various infections [2]. Most of the bioactive metabolites from these plants especially flavonoids demonstrated potent antioxidant activity in vitro and in vivo [3]. Many synthetic antioxidants and metal chelator components have also exhibited toxic or mutagenic effect coupled with suppression of body immunity which have shifted attention towards naturally occuring antioxidants [4]. *Alpina muricata* is grown specifically for its essential oils in its leaves and barks where thymol, eugenol, citral, geraniol and linalool have been extracted [5]. Medicinal plants play pivotal role in the health care of ancient and modern cultures, Indians and Chinese system of medicine depend solely on plant based drugs to treat various human ailments since they contain different components of therapeutic value [6]. Besides, plant based drugs still relevant and remain very important source of therapeutic agents due to availability, relatively cheaper cost as well as non-toxic nature compared to unorthodox medicine [7]. Most medicinal plants contain antioxidant compounds that protect the cells against the damaging effects of reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide [8]. These radicals are generated in human body via aerobic respiration or rather from exogenous sources and thus play crucial roles in the development of various ailments such as arthritis, asthma, cardiovascular disorders, neurodegenerative and parkinson diseases. However, phenolic compounds from medicinal plants possess strong antioxidant activity and may help protect the cells against oxidative assault caused by these free radicals [9]. Hence, this study was designed to investigate and evaluate the antioxidant potential of Alpina muricata leaf extract using the aforementioned antioxidant indices.

II. Materials And Methods

Collection of Plant Sample

Fresh leaves of *Alpina muricata* plant were fetched from a reserved virgin forest neear Ikare-Akoko township, Ondo-State, Nigeria. The plant was authenticated at the herbarium centre of Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Preparation of Plant Extracts

The aqueous extract was prepared by extracting 150g of powdered sample in cold sterile distilled water, aggitated with mechanical shaker, and filtered via buchner funnel with No 1 whatman's filter paper, frozen at -40^0C and dried with freeze dryer for 72hrs. The percentage yield of 11.33% was obtained [10] for aqueous

extract while 120g each of powdered sample was extracted with (70%) ethanol and ethylacetate respectively.. The mixture was decanted and filtered with No 1 Whatman's filter paper which measured up to 600mls and was evaporated to dryness to both give 9.96% yield.

DPPH Radical Scavenging Assay

10µl of plant leaf extracts was added to 100µl of DPPH solution in a microtitre plate. The reaction mixture was incubated at 25^0C for 5mins and was left in the dark for 30mins after which the absorbance was read at 520nm. DPPH with corresponding solvents without plant extract served as control while methanol with corresponding plant extracts served as blank and was calculated as……

$$\% \text{ Inhibition of DPPH} = \frac{\text{Absorbance}_{\text{control}} \text{ --- } \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} \text{ X } 100$$

The free radical scavenging ability of leaf and flower extracts of *Alpina muricata* plant was determined by 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) using method described by [11]. DPPH is a protonated radical with maximum absorption at 517nm that decreases with the scavenging of the proton radicals by plant extracts. It is a commercially available stable free radical, purple in colour where the antioxidant molecules in the extracts react with DPPH when incubated and thus convert it into di-phenyl hydrazine, which is yellow in colour. The degree of decolouration of purle to yellow was measured at 520nm which is a measure of scavenging potential of plant extracts.

Metal Chelation Assay

The metal chelating ability of the extracts was determined using a modified method of (12). Freshly prepared 500µmolL^{-1} FeSO4 (150µL) was added to a reaction mixture containing 168µL of 0.1molL^{-1} Tris HCl at pH 7.4, 218µL saline solution and 25µL of extracts. The reaction mixture was incubated for 5min. before the addition of 13µL of 0.25 1,10-phenanthroline (w/v). The absorbance was measured at 510nm in spectrophotometer and the metal chelating ability was calculated with the formula below:

$$\% \text{ Chelation} = \frac{\text{Absorbance}_{\text{control}} \text{ ----- } \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} \text{ x } 100$$

III. Results

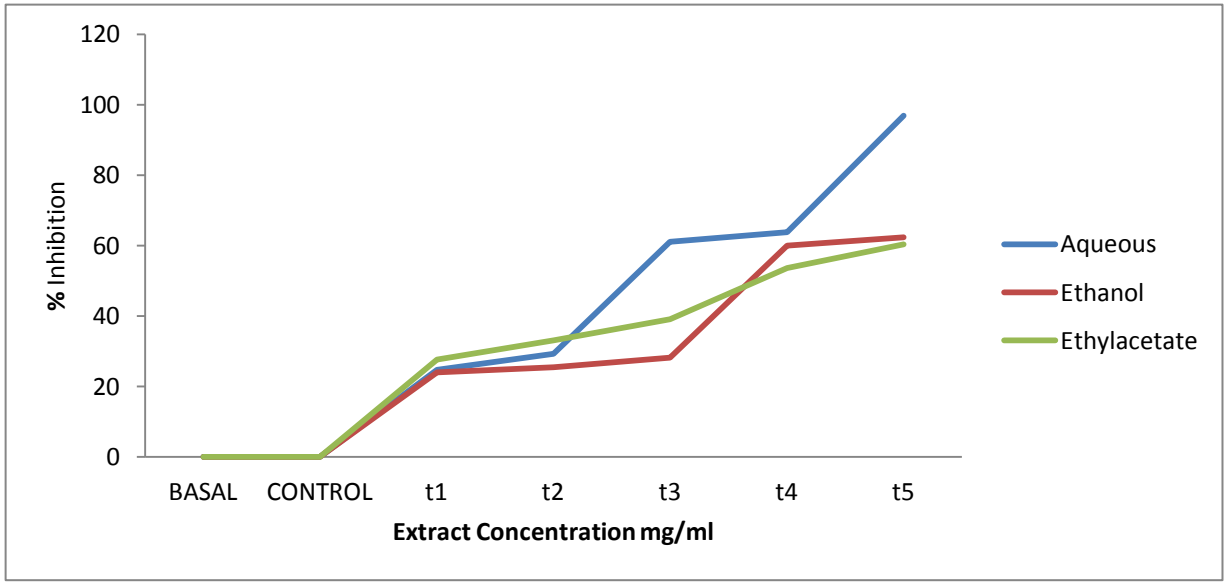

Fig.1: DPPH free radical scavenging ability of ethanolic, ethylacetate and aqueous leaf extracts of *Alpina muricata*

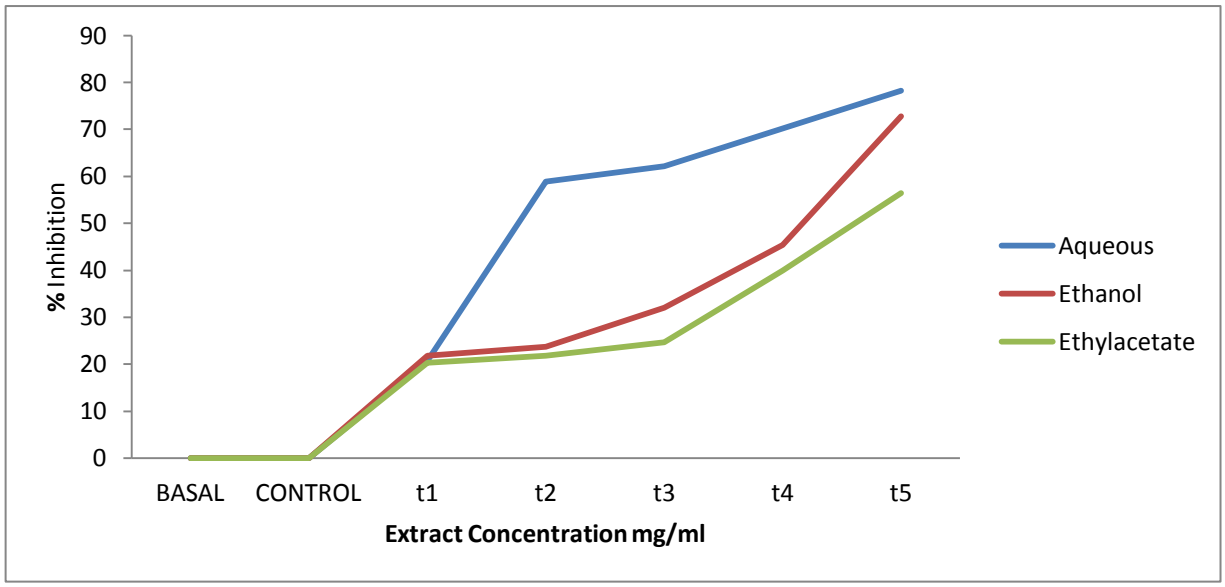

Fig. 2: Metal chelating ability of ethanolic, ethylacetate and aqueous leaf extracts of *Alpina muricata*

IV. Discussion

Free radicals are constantly generated resulting in extensive damage to tissues and macromolecules leading to development of various diseases. Medicinal plants are employed as alternative therapy to mitigate the oxidative stress related diseases [13]. Results from this study showed that DPPH scavenging ability of *alpina muricata* leaf screened in aqueous, ethanolic and ethylacetate solvent system significantly ($P<0.05$) demonstrated strong DPPH inhibition. The leaf extract demonstrated highest DPPH inhibition at (97%) aqueous solvent system while the lowest DPPH inhibition (56%) was obtained in ethylacetate solvent as depicted in **Figure 1** above. The high antioxidant capacity of the plant extracts may be attributed to the hydrogen donating ability of its inherent phenols and flavonoids [14]). DPPH is a stable free radical in aqueous and organic medium usually used as a substrate to evaluate the antioxidative activity of antioxidant [15]. It accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Besides, the metal chelating ability of the extract tested in the three solvents showed that aqueous extract demonstrated the strongest metal chelating potential with highest chelating ability (80%) while ethylacetate extract demonstrated the least metal chelating potential at (50%). Metal ions play an important role in the acceleration of oxidation of essential biological molecules. They usually catalyze the formation of free radicals that lead to the propagation of reactive oxygen species (ROS) in lipid peroxidation (16). This however, indicates that the chelation potential demonstrated by the extracts on Fe^{2+} is capable to protect the cell against oxidative damage. Moreover, it was reported that chelating agent such as synthetic chelators which form d-bond with metals are effective as secondary antioxidant. They usually reduce the redox potential, thereby stabilizing the oxidized form of the metal ions (17). In addition, chelating agent inhibits radical mediated oxidative chain reactions in biological system and thus, improve human health, stability and safety as well as improve food quality. Citric acid and EDTA are among most commonly used chelators, while plant phenolic compounds have also been found to be good metal chelators (18).

V. Conclusion

It can be inferred from the data obtained in **Figure 1 and 2** above that *Alpina muricata* plant leaf possesses higher antioxidant potentials which are of greater medicinal values.

CONPLIANCE WITH ETHICAL STANDARD

The plant materials used were authenticated and permitted by Herbarium Centre of Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

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DECLOSURE OF CONFLICT OF INTEREST

No conflict of interest

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