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In vitro antioxidant and anti-arthritic effect of the aqueous and ethanolic leaf and root bark extract of *Alafia barteri*

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ABSTRACT

Alafia barteri (Apocynaceae) is a climbing shrub having white or pink flowers. Traditionally, it has been used to treat diseases like malaria, sickle cell anemia, and eye infections. This research is focused on investigating the antioxidant and anti-arthritic activities of the aqueous and ethanol leaf and root extract of *Alafia barteri* plant in vitro. *In-vitro* antioxidant methods used were 2, 2 -diphenyl-1-picrylhydrazyl assay, reducing power activity and hydrogen peroxide scavenging assay while the anti-arthritic activity was studied using the assay method of protein denaturation. Results revealed that aqueous and ethanol root extracts scavenge free radicals, thus inhibiting damage caused by oxidative stress in arthritis while the ethanol extracts of both the leaf and roots had good anti-arthritic activities as seen in its ability to decrease protein denaturation.

Keywords: *Alafia barteri*, Anti-arthritic, Anti-oxidant, Denaturation.

INTRODUCTION

Medicinal plants are plants that produce biologically useful substances in some of its parts which may be used for healing purposes or substances that serve as raw materials for further drug manufacturing. Use of medicinal plants have become very common in the treatment of different ailments in the modern world, thus raising their risk of extinction or loss of genetic diversity [18].

Currently, several diseases which affect human heart, the nervous system and human metabolism are on the rise. Among the metabolic disorders, arthritis is one of the oldest, which primarily affects the joints.

Rheumatoid arthritis (RA) is an inflammatory disease which persistently affects the joints, ultimately leading to pain, disability and death. It is commonly triggered by dysfunction of normal immune response which is characterized by loint inflammation and continuous caetligae desctruction [19]. About one percent of the general population is affected by this disease [3]. RA is a systemic disease as it does not affect only the joints. The occurrence of RA can lead to the damage of other body organs like the eyes, lungs and heart, ultimately leading to premature death. Several studies have shown that the onset and progression of RA is associated with a negative balance in the pool of oxidative molecules, leading to more production of pro-oxidants, that is free, unstable and highly reactive radicals. However, excess concentrations of these reactive species cause cellular damage and as a result, antioxidant therapies are promising in the treatment of this oxidative stress related disease. Many studies have also confirmed that one of the causes of rheumatoid arthritis is protein denaturation which is linked to the development of auto-antigens.

Common conventional modern drugs (NSAIDS) has only been found to minimize the symptoms, however, they still produce many undesirable renal, liver and hematological effects in the body.

Alafia barteri(Apocynaceae) is commonly known as agbari etu(instant fever remedy) in Yoruba language, loko or mende (Sierra leone), anyi (ivory coast), akan-asante or fante (ghana), obompa, ota nza (igbo) [14]. Its effectiveness in the African traditional medicine system has prompted its use in the treatment of sickle cell anaemia, eye infections and toothache [17]. Leaf extracts of the plant were found to have antibacterial and antifungal activities [1, 9], anti-plasmodial activity [14], antidiabetic activity [3]. Its root and stem extract has also been shown to possess analgesic and anti-proliferative activities [11,10]. Adelakun *et al*., 2018 has also reported its effect on spermatogenesis and steroidogenesis. This present research focuses on examining the antioxidant and anti-arthritic effects of the aqueous and ethanol leaf and root extracts of *Alafia barteri* plant in-vitro, thus providing a scientific basis for its traditional use and also to explore new biological and pharmacological activities of this plant.

MATERIALS AND METHODS

Plant Collection

Alafia barteri leaves and root barks were harvested from Ikire in Osun State. The plant materials were identified in the department of Botany University of Lagos where it was given the voucher number LUH8657.

Extract preparation

Leaves and Root bark after air drying for two weeks were ground to powder using the blender while the root bark was chopped into small pieces.100g of each ground and chopped part (that is, leaf and root bark) was mixed with 500mls of distilled water and ethanol respectively and was left for 72hours at room temperature with intermittent stirring. The mixtures were filtered with Whatman no 1 filter paper and the filtrates were collected separately in a clean beaker. The extracts were evaporated, using laboratory oven at 45^0C to obtain a dark viscous extract.

Equipment and Reagents

Reagents used in this study were obtained from the Institution's laboratory, Ethanol**,** Phosphate buffer**,** Potassium ferricyanide**,** Trichloro acetic acid**,** Ferric chloride**,** Ascorbic acid**,** Hydrogen peroxide**,** Methanol**,** 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Hydrochloric acid**,** Bovine serum albumin**,** Aspirin and Diclofenac (purchased from a pharmaceutical store in Mowe, Ogun State, Nigeria.)

In-vitro antioxidant assays

Reducing power Assay

The previously published method of Jayanthi *et al*, 2011 was used to determine the invitro reducing power of the extracts where 50-300ug/ml of the plant extracts was prepared respectively. 2.5mls each of phosphate buffer and potassium ferricyanide were added and the solutions were allowed to remain for 20mins at 50 ºC in the water bath. The solutions were allowed to cool after which 2.5mls of 10% trichloroacetic acid was added, Centrifugation was done at 3000rpm for 10minutes after cooling. 2.5mls of supernatant obtained after centrifugation was retrieved and added to a test-tube containing distilled water and ferric chloride. The standard used was ascorbic acid while the control was prepared in similar manner excluding samples. The absorbance was measured at 700 nm. The reducing power activity was calculated using the formula:

$$\% \text{ increase in reducing power} = \frac{\text{A test} - \text{A blank}}{\text{A blank}} \times 100$$

Hydrogen peroxide scavenging activity

The ability of *Alafia barteri* leaf and root bark extract to scavenge hydrogen peroxide radicals was evaluated according to the method of Dehpour *et al*, 2009. 1 ml of hydrogen peroxide solution (prepared in phosphate buffer of ph 7) was added to 0.1mg/ml of each extract. A blank solution which contains phospahate buffer only was also prepared and the absorbance at 560nm was read with the UV spectrophotometer. Experiment was repeated in triplicates.

The given formula was used to calculate the percentage of hydrogen peroxide radicals mopped up by the extracts.

$$\text{Percentage scavenged } [H_2O_2] = 1 - \frac{\text{Abs (standard)}}{\text{Abs (control)}} \times 100$$

Abs control is the absorbance of the control (without extract) at560nm;

Abs sample is absorbance in the presence of the extract at 560nm.

DPPH Radical Scavenging Activity

The DPPH Scavenging activity was estimated according to the method of Brand-Williams *et al*,1995. A methanol solution of DPPH(2,2-diphenyl-1-picrylhydrazyl) (0.1mM) was firstly prepared and 0.2mls of each plant extract was added to 3mls of the solution. Absorbance was read at 517nm as color change took place which was indicative of the scavenging activity. The experiment was repeated using ascorbic acid as the standard

The percentage of inhibition was calculated with the formula:

$$\% \text{ antioxidant capacity} = [Ac - As / Ac] \times 100$$

Where: Ac = absorbance of control and
As = absorbance of sample.

In-vitro anti-arthritic activity

Inhibition of protein denaturation method

This assay was carried out according to the method of KarB, 2012. In this experiment, four sets of solutions were prepared:

Test solutions contained 0.45 ml of BSA (bovine serum albumin) (5% w/v) and 0.05 ml different concentrations of the plant extract (50-300ug/ml)

Test control solution contained 0.45 ml of BSA (5% w/v) and 0.05 ml distilled water

Product control solution contained 0.45 ml distilled water and 0.05 ml of plant extract (different concentrations

Standard solution contained 0.45 ml BSA (5% w/v) and 0.05 ml diclofenac sodium (500 µg/mL) which serves as the standard drug.

All solutions prepared were adjusted with 1N hydrochloric acid to pH 6.3. The samples were maintained for 20 min at 37˚C. The temperature was raised to 57°C for 3 min and allowed to cool. 2.5mls of phosphate buffer saline was added and the absorbance at 416 nm was read.

Experiment was performed in triplicates and results were compared with the standard drug. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition of protein denaturation} = 1 - \frac{\text{O.D of test solution} - \text{O.D of product control}}{\text{O.D of test control}} \times 100$$

RESULTS AND DISCUSSION

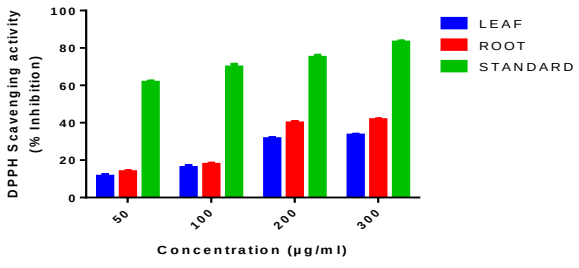

Figure 1: DPPH Scavenging activity of the aqueous leaf and root extract of *Alafia barteri*

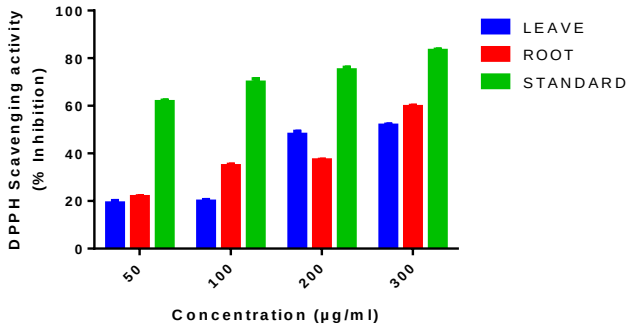

Figure 2: DPPH Scavenging activity of the ethanol leaf and root extract of *Alafia barteri*

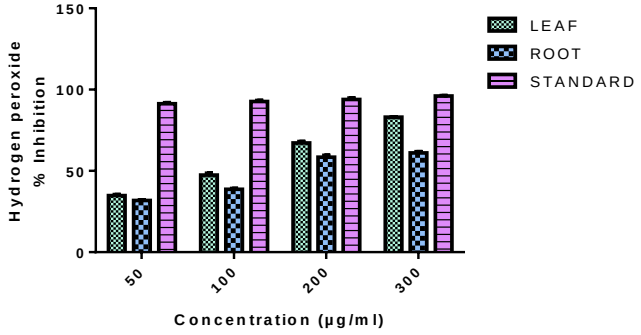

Figure 3: Hydrogen Peroxide scavenging activity of aqueous leaf and root extract of *A. barteri*

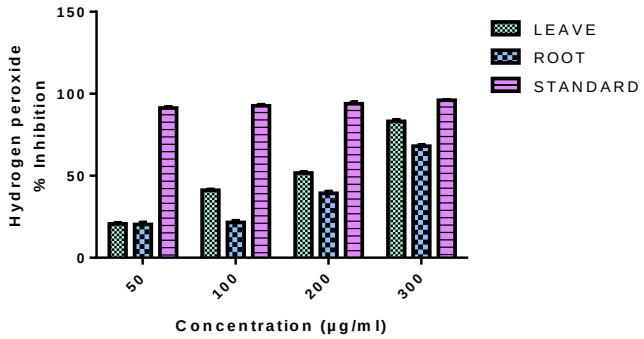

Figure 4: Hydrogen Peroxide scavenging activity of ethanol leaf and root extract of *A. barteri*

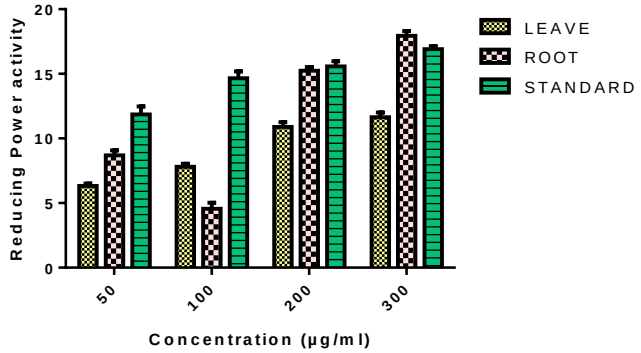

Figure 5: Reducing power activity of the aqueous leaf and root extract of *A. barteri*

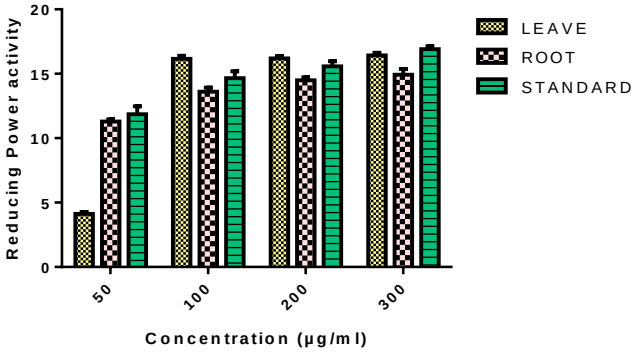

Figure 6: Reducing power activity of the ethanol leaf and root extract of *A. barteri*

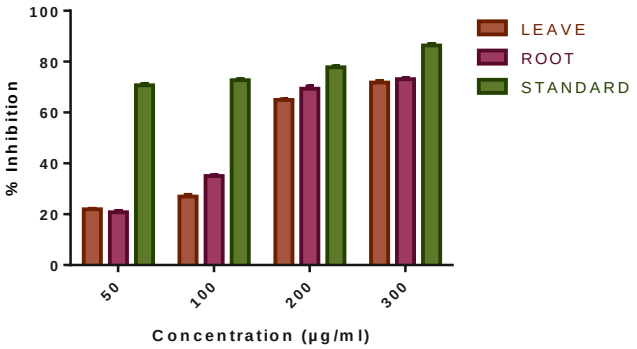

Figure 7: Effect of aqueous leaf and root extract of *A.barteri* on protein denaturation using egg albumin

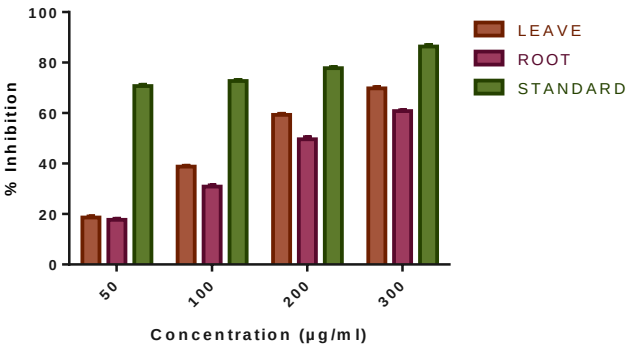

Figure 8: Effect of ethanol leaf and root extract of *A.barteri* on protein denaturation using egg albumin

Medicinal plants having antioxidant and anti-arthritic properties have been on the rise owing to their ability to treat diverse diseases. They serve as alternative sources of agents with significant anti-arthritic and antioxidant activity due to the risk of adverse effects experienced when using synthetic agents.

DPPH scavenging activity is a simple and commonly used method to test natural compounds or plant extracts for in-vitro antioxidant activity. DPPH, purple in colour, is a stable free radical at room temperature. Its potential to minimize the absorption of an electron or a hydrogen radical from antioxidants can be evaluated by reduction in absorption at 517nm

In this analysis, DPPH radical scavenging activity of the aqueous and ethanol leaf and root extract of *Alafia barteri* was measured and it was revealed that Ascorbic acid standard possessed higher activity than the extracts at their respective concentrations as seen in their inhibition percentage which also increased as the concentration increased. The aqueous root extract of the plant had higher activity compared to the aqueous leaf extract while in ethanol extract the leaves have the highest percentage inhibition after the standard (Figure 1).

Hydrogen peroxide scavenging activity as seen in figures 3 and 4 were expressed as percentage inhibition. The aqueous and ethanol leaf extracts had higher percentage inhibition compared to other extracts though the standard still had the highest. Figure 5 and 6 reveals that the reducing power of the extracts was dependent on their concentration however, the ethanol leaf and root extracts had higher activity. No significant difference was observed between the percentage inhibition of protein denaturation by the aqueous leaf and root extract, however the ethanol leaf extract had higher percentage than the root extract. (figure 7 and 8)

H_2O_2 is extremely essential since it is capable of penetrating biological membranes. H_2O_2 can often become toxic to cells due to its ability to produce hydroxyl radicals (OH-) [8]. Phenolics that can donate electrons to H_2O_2 can be attributed to H_2O_2 scavenging by the extract, thereby neutralizing Hydrogen to water (H_2O). The results show that *Alafia barteri's* aqueous and ethanol leaf and root extract had a strong H_2O_2 scavenging activity that could be attributed to the presence of antioxidant compounds. Since good electron donors are the antioxidant components present in the extracts, they can speed up the conversion of H_2O_2 to H_2O. The results indicate a good inhibition percentage that increases as the extract concentration increased, with a high scavenging activity of the standard drug. The standard for each extract have the highest percentage inhibition followed by the aqueous leaf and also ethanolic leaf.

The presence of antioxidants in the aqueous and ethanol leaf and root-bark extract of *Alafia barteri* in the reducing power assay can lead to a reduction in the ferricyanide complex form. The antioxidant activity of a compound can be determined once its reducing power is determined [16]. The reducing power of the aqueous and ethanol leaf and root extract of *Alafia barteri* was determined by comparing with that of ascorbic acid. It was observed that as the extract concentration increased, their reducing powers also increased. At 300 µg/ml, the aqueous extract of the root has the highest percentage inhibition than that of the standard while in ethanol extract the leaves have the highest percentage inhibition. This implies that these extracts are able to react significantly to free radicals in order to turn them into more stable non-reactive species and to end radical chain reactions.

The protein denaturation assay method was used for evaluating the in-vitro anti-arthritic properties of the aqueous and ethanol leaf and root-bark extract of *Alafia barteri* with a wide range of dose concentrations. Results showed that inhibition of protein denaturation by the extracts and the standard drug, diclofenac sodium depended on their respective concentrations (50-300µug/ml)

The aqueous leaf and root extracts almost inhibited protein denaturation equally with no significant difference.

It has been stated that protein denaturation plays a role in the progression of rheumatoid arthritis. Some inflammatory auto-immune diseases are characterized by the production of auto-antigens as a result of protein denaturation.[4].Denaturation mechanisms are likely to involve changes in electrostatic, hydrogen, hydrophobic and disulphide bonding. It can be stated from the results of this study that aqueous and ethanol leaves and root parts of *Alafia barteri* are capable of regulating auto antigen production and inhibiting protein denaturation in rheumatic diseases [5].

The free radical scavenging property of *Alafia barteri* may be one of the mechanisms by which this plant is effective. The use of *Alafia barteri* plant may be beneficial for the prevention of degenerative diseases associated with oxidative stress.

CONCLUSION

This study reveals that the aqueous and ethanol leaf and root extract of *Alafia barteri* possesses radical scavenging and anti-arthritic activities as determined by the inhibition of protein denaturation assay, DPPH scavenging, reducing power and hydrogen peroxide scavenging assay.

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