



## **Anti-oxidative and Anti-inflammatory Potential of Aqueous and Ethanol Extract of Leaves and Roots of *Napoleona imperialis***

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author EOE designed the study. Author UEB performed statistical analysis. Author BUE proof read the final work while authors SS and NAC wrote the first draft. All authors read and approved the final manuscript.*

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### **ABSTRACT**

*Napoleona imperialis* is a wild plant commonly found in South eastern part of Nigeria which is mostly used for the treatment of wounds. The aim of this research was to evaluate the *in vitro* anti-oxidative and anti-inflammatory potential of aqueous and ethanol extracts of leaves and roots of *Napoleona imperialis*. The DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging potential of the extracts were evaluated with ascorbic acid as standard. Lipoxidase activity, membrane stabilization and percentage inhibition of acetylcholinesterase was assayed using spectrophotometric method with reference drugs as standard. The DPPH radical scavenging potential of the aqueous leaf extract was observed to be maximum at a concentration of 150 µg/ml which is similar to the result obtained for

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ascorbic acid. There was a statistically significant difference in the ABTS radical scavenging potential of the ethanol leaf extract ( $23.67 \pm 0.61$ ) and ethanol root extract ( $32.54 \pm 0.84$ ) at  $10 \mu\text{g/ml}$  when compared with ascorbic acid ( $9.90 \pm 0.26$ ). The ethanol leaf extract showed a significantly higher percentage inhibition of lipoxidase activity at  $100 \mu\text{g/ml}$  ( $20.88 \pm 0.77$ ) and  $150 \mu\text{g/ml}$  ( $31.20 \pm 0.80$ ) when compared to reference drug ( $26.28 \pm 0.68$  and  $31.67 \pm 0.82$  respectively). Furthermore, the ethanol leaf extract demonstrated a percentage membrane stabilization activity of  $41.78 \pm 1.08$  and  $43.65 \pm 1.12$  at  $20 \mu\text{g/ml}$  and  $50 \mu\text{g/ml}$  respectively compared with the standard drug diclofenac ( $38.35 \pm 0.99$  and  $41.86 \pm 1.08$  respectively). The present study therefore suggests that extract of *N. imperialis* has good anti-oxidative and anti-inflammatory potential and could be ameliorated in diseases due to inflammation and oxidation.

**Keywords:** Anti-oxidant; anti-inflammatory; *Napoleona imperialis*; ascorbic acid; inflammation.

## 1. INTRODUCTION

Herbal medicine has become a subject of global importance, making an impact on both world health and international trade. Medicinal plants continue to play central roles in the healthcare system of large proportion of the world's population. This is particularly true in the developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations [1]. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals, health care, adverse effects that follow their use and the cultural and spiritual point of view of the people of the countries [1].

*Napoleona imperialis* is a small evergreen tropical West African tree. The plant belongs to the family Lecythidaceae and is variously known as Ntum in Ikwano of Ibo language, Ukpakonrisa in Edo, Obu-anagbo and Otukuche in Igala, Isi efe in Umuahia, and Akpodo in Awka,

all in Nigeria. It grows to about 6m with a dense low branching crown and is found in Benin Republic, Nigeria, Gabon, Democratic Republic of Congo, and Southward of Angola [2]. Fig. 1. is a pictorial representation of the leaves and roots of *N. imperialis*.

Several reports have shown that *N. imperialis* has wound healing [3], anti-hypertensive [4], anti-inflammatory [5], anti-diarrheal and anti-ulcerative activity [6], free radical scavenging activity [6] and abortifacient properties [3]. The phytochemicals present in the leaves have been reported to include cyanide, glycosides, tannins, proteins, and saponins [7]. The roots of *N. imperialis* have been reported to contain important phytochemicals such as alkaloids, flavonoids, tannins, steroids, glycoside, saponins, carbohydrate, resin and proteins, and possess anti-oxidant and free-radical scavenging properties [7].

In view of the various medicinal applications of the root and leaf of *N. imperialis*, the present study is designed to evaluate the *in vitro* antioxidant and anti-inflammatory potential of the root and leaf of the plant.



**Fig. 1.** Leaves and roots of *N. imperialis*

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Leaves and roots of *Napoleona imperialis* were obtained from Mgbirichi community in Imo State and were identified at the herbarium of University of Nigeria, Nsukka.

### 2.2 Preparation of Extracts

The leaves and the roots of *Napoleona imperialis* were washed clean to remove all debris and then air dried at room temperature to remove water content. After complete drying, the leaves and roots were ground to powder using a manual grinder. 200 g each of the leaf and root powder was macerated in 80% ethanol for 48 hours. The filtrate was obtained and concentrated in a water bath at 40°C to obtain the crude extracts. The crude extracts were preserved in a refrigerator at 4°C till they were used for the study.

### 2.3 *In vitro* Anti-oxidant and Anti-inflammatory Assays

The *in vitro* anti-oxidant and anti-inflammatory assays were performed with the crude extracts. The assays were performed in triplicates. IC50 values (concentration at which there is 50% enzyme inhibition) of best samples were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

### 2.4 Determination of Antioxidant Activity

The free radical scavenging activity of the aqueous and ethanol leaf and root extracts of *N. imperialis* was determined by using *in vitro* assays such as DPPH free radical assay and ABTS free radical assay. DPPH free radical scavenging and ABTS radical scavenging assay was carried out according to standard methods. Vitamin C was used as the standard antioxidant for comparison.

### 2.5 Determination of Anti-inflammatory Activity

The different assays used in this study to evaluate anti-inflammatory activity of the extracts include lipoxigenase inhibitory activity, acetyl cholinesterase inhibitory activity and RBC membrane stabilization activity. Lipoxigenase inhibitory activity was assayed according to the

method of Tappel [8] with slight modifications by Wallace and Wheeler [9]. Assay of acetyl cholinesterase inhibitory activity was performed by spectrophotometric method described by Ellman et al. [10]. RBC Membrane stabilization activity of the extracts was assayed according to the method described by Chowdhury et al. [11]. Human red blood cell from voluntary donor was used for the assay. Diclofenac and galantamine were used as standard anti-inflammatory agents for comparison with the extracts.

### 2.6 Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS package and values were considered significant at  $p < 0.05$  compared to one another.

## 3. RESULTS

### 3.1 *In vitro* Antioxidant Activity of Extracts of *N. imperialis* Root and Leaf

#### 3.1.1 Percentage DPPH free radical scavenging activity

The percentage DPPH free radical scavenging activity of ethanol and aqueous extracts of root and leaf of *N. imperialis* is presented in Table 1.

#### 3.1.2 Percentage ABTS oxide radical scavenging

The Percentage ABTS oxide radical scavenging activity of ethanol and aqueous extracts of root and leaf of *N. imperialis* is presented in Table 2.

### 3.2 *In vitro* Anti-inflammatory Activity of Extracts of *N. imperialis* Root and Leaf

#### 3.2.1 Percentage inhibition of lipoxigenase activity

The lipoxigenase inhibitory activity of ethanol and aqueous extracts of root and leaf of *N. imperialis* is presented in Table 3.

#### 3.2.2 Percentage inhibition of acetylcholinesterase activity

The acetylcholinesterase inhibitory activity of ethanol and aqueous extracts of root and leaf of *N. imperialis* is presented in Table 4.

**Table 1. Percentage DPPH radical scavenging of extracts of *N. imperialis* leaf and root**

Samples / Concentration	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	IC50
Vitamin C	48.37 ± 1.25	52.33 ± 1.35	60.14 ± 1.55	66.83 ± 0.99	68.76 ± 1.77	56.99 ± 1.47
Aqueous Leaf Extract	48.37 ± 1.25	52.33 ± 1.35	60.14 ± 1.55	66.83 ± 0.99	68.76 ± 1.77	56.99 ± 1.47
Aqueous Root Extract	1.35 ± 0.03 <sup>*</sup>	2.59 ± 0.07 <sup>*</sup>	3.85 ± 0.10 <sup>*</sup>	14.83 ± 0.22 <sup>*</sup>	15.68 ± 0.40 <sup>*</sup>	416.98 ± 10.74 <sup>*</sup>
Ethanol Leaf Extract	4.11 ± 0.11 <sup>*</sup>	11.20 ± 0.29 <sup>*</sup>	22.31 ± 0.57	47.93 ± 0.71	49.88 ± 1.29	126.25 ± 3.25
Ethanol Root Extract	-0.97 ± 0.02 <sup>*</sup>	3.67 ± 0.09 <sup>*</sup>	7.25 ± 0.19 <sup>*</sup>	22.53 ± 0.34	25.78 ± 0.66	235.58 ± 6.07

The values are presented as Mean ± Standard Error of Mean. \* = statistically significant when compared to Vitamin C at  $p < 0.05$

**Table 2. Percentage ABTS oxide radical scavenging of extracts of *N. imperialis* leaf and root**

Samples / Concentration	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	IC50
Vitamin C	9.90 ± 0.26	20.12 ± 0.52	24.19 ± 0.62	28.03 ± 0.72	36.66 ± 0.94	219.49 ± 5.66
Aqueous Leaf Extract	14.19 ± 0.37	16.65 ± 0.43	24.32 ± 0.63	24.52 ± 0.63	28.26 ± 0.73	354.59 ± 9.14
Aqueous Root Extract	6.35 ± 0.16	9.96 ± 0.26	18.42 ± 0.47	17.76 ± 0.46	17.54 ± 0.45	546.16 ± 14.07
Ethanol Leaf Extract	23.67 ± 0.61 <sup>*</sup>	23.96 ± 0.62	32.97 ± 0.85	35.31 ± 0.91	40.33 ± 1.04	208.86 ± 5.38
Ethanol Root Extract	32.54 ± 0.84 <sup>*</sup>	-16.37 ± 0.42 <sup>*</sup>	11.07 ± 0.29 <sup>*</sup>	-36.94 ± 0.95	-6.59 ± 0.17 <sup>*</sup>	--

The values are presented as Mean ± Standard Error of Mean. \* = statistically significant when compared to Vitamin C at  $p < 0.05$

**Table 3. Percentage inhibition of lipoxygenase activity of extracts of *N. imperialis* leaf and root**

Samples / Concentration	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	IC50
Diclofenac	8.98 ± 0.23	15.55 ± 0.40	17.45 ± 0.45	26.28 ± 0.68	31.67 ± 0.82	41.48 ± 1.07 <sup>*</sup>
Aqueous Leaf Extract	1.29 ± 0.033 <sup>*</sup>	3.97 ± 0.10 <sup>*</sup>	9.39 ± 0.24 <sup>*</sup>	12.86 ± 0.33	16.93 ± 0.44	401.00 ± 10.33
Aqueous Root Extract	7.00 ± 0.18	8.24 ± 0.21	10.01 ± 0.26	15.07 ± 0.39	13.91 ± 0.36 <sup>*</sup>	471.69 ± 12.15
Ethanol Leaf Extract	3.85 ± 0.10	9.08 ± 0.23	14.75 ± 0.38	29.88 ± 0.77	31.20 ± 0.80	162.16 ± 4.18
Ethanol Root Extract	3.29 ± 0.08	8.09 ± 0.21	8.78 ± 0.23 <sup>*</sup>	9.49 ± 0.24 <sup>*</sup>	10.63 ± 0.27 <sup>*</sup>	2343.61 ± 60.39 <sup>*</sup>

The values are presented as Mean ± Standard Error of Mean. \* = statistically significant when compared to Diclofenac at  $p < 0.05$

**Table 4. Percentage inhibition of acetylcholinesterase activity of extracts of *N. imperialis* leaf and root**

Samples / Concentration	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	IC50
Diclofenac	37.83 ± 0.97	38.57 ± 0.99	41.86 ± 1.08	47.68 ± 1.23	54.32 ± 1.40	102.69 ± 2.65
Aqueous Leaf Extract	-7.01 ± 0.18	4.03 ± 0.10	30.52 ± 0.79*	33.65 ± 0.87*	43.95 ± 1.13*	149.43 ± 3.85
Aqueous Root Extract	4.70 ± 0.12	11.64 ± 0.30	14.70 ± 0.38	28.28 ± 0.73*	37.08 ± 0.96*	190.53 ± 4.91
Ethanol Leaf Extract	32.75 ± 0.84	41.78 ± 1.08	43.65 ± 1.12	44.39 ± 1.14*	52.30 ± 1.35*	106.30 ± 2.74
Ethanol Root Extract	3.58 ± 0.09	7.01 ± 0.18	7.46 ± 0.19	39.25 ± 1.01*	40.74 ± 1.05*	177.72 ± 4.58

The values are presented as Mean ± Standard Error of Mean. \* = statistically significant when compared to Diclofenac at  $p < 0.05$

**Table 5. Percentage membrane stabilization activity of extracts of *N. imperialis* leaf and root**

Samples / Concentration	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	IC50
Galantamine	7.03 ± 0.18	12.90 ± 0.33	17.26 ± 0.44	22.65 ± 0.58	26.17 ± 0.67	312.94 ± 8.06
Aqueous Leaf Extract	2.62 ± 0.07	3.34 ± 0.09	3.84 ± 0.10	6.84 ± 0.18	8.11 ± 0.21	1144.96 ± 29.50*
Aqueous Root Extract	3.18 ± 0.08	3.39 ± 0.09	7.70 ± 0.20	7.54 ± 0.19	7.04 ± 0.18	6081.59 ± 156.7#
Ethanol Leaf Extract	3.93 ± 0.10	4.93 ± 0.13	5.95 ± 0.15	6.07 ± 0.16	6.62 ± 0.17	3812.49 ± 98.23*
Ethanol Root Extract	1.60 ± 0.04*	1.89 ± 0.05*	2.97 ± 0.08*	3.17 ± 0.08*	3.58 ± 0.09*	3411.33 ± 87.90*

The values are presented as Mean ± Standard Error of Mean. \* = statistically significant when compared to Galatamine at  $p < 0.05$

### 3.2.3 Percentage membrane stabilization activity

The membrane stabilization activity of ethanol and aqueous extracts of root and leaf of *N. imperialis* is presented in Table 5.

## 4. DISCUSSION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. In a biological system, they protect cells from the damage caused by unstable molecules known as free radicals [12]. Plants are important source of potential compounds for the development of new therapeutic agents. Plant phenolic are widely distributed in the tissues of plants as well as play a vital role in the highly effective free radical scavenging and antioxidant activity of plants. Thus, the antioxidant capacity of a plant sample can be attributed mainly to its phenolic compounds [7].

The DPPH free radical scavenging assay has been largely used as a quick, reliable and reproducible parameter to search for the in vitro antioxidant activity of pure compounds as well as plant extracts [13,14]. In the present study, the DPPH radical scavenging activity of the plant extracts increased with an increasing concentration which was found to be statistically similar to that of the ascorbic acid standard. Similar findings have been observed by other authors [7,6,15]. The ethanol and aqueous root extracts showed lower DPPH radical scavenging activities when compared to ethanol and aqueous leaf extract. The Aqueous leaf extract exhibited higher DPPH radical scavenging activity which was statistically significant ( $p < 0.05$ ) when compared with Vitamin C.

The scavenging of ABTS free radical by the extracts was found to be higher than that of DPPH. Several factors like stereo-selectivity of the radicals or the solubility of the extracts in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals [16]. Similarly, Wang et al. [17], reported that some compounds that have ABTS<sup>•+</sup> scavenging activity could not scavenge DPPH. This further showed the potential of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating free radical-related pathologic damage. Studies have reported that methanol extract of *Napoleona imperialis* has antioxidant properties by

scavenging free radicals, and also decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzyme levels [7].

In the present investigation, leaf and root extracts of *N. imperialis* exhibited outstanding DPPH and ABTS scavenging activities. The qualitative phytochemical analysis carried out on *Napoleona imperialis* root extract [6] and leaf extract [18] showed the presence of flavonoids, tannins and saponins as the major phytochemical constituents present in relatively high amount. It has been reported that high phenolic content of plant extracts could be responsible for their antioxidant activity [19,20]. It is therefore possible that the phenolic and flavonoids present in the extracts may be responsible for the observed antioxidant activity in this present study.

Leukocyte infiltration occurs during inflammatory response due to its role in defense against inflammation. These cells release their lysosomal contents, such as bactericidal enzymes and protease, which cause further tissue damage and inflammation. Injury to the cell membrane increases the cells' susceptibility to secondary damage by the free radicals produced by lipid peroxidation. As the RBC membrane is similar in characteristics to the lysosome membrane, the inhibition of RBC hemolysis is used to measure the anti-inflammatory activity of medicinal substances, including plant extracts.

In the present study, the *N. imperialis* ethanol extract exhibited higher ability to stabilize RBC membrane than diclofenac, indicating that the *N. imperialis* ethanol extract may prevent the lysis of the RBC membrane. Since the membranes of RBC and lysosome share similar properties, the membrane-stabilizing effect of the ethanol root extract may also inhibit the release of the PLA2 enzyme, which plays an important role in inflammatory processes. Therefore, this extract could become a potential therapeutic agent to treat human inflammatory diseases [21].

The lipoxygenase group of enzymes (5, 8, 12, and 15 LOX) plays a role in various inflammatory disorders. The isomeric enzyme 15-LOX is a key enzyme involved in the synthesis of leukotrienes from arachidonic acids. Biologically active leukotrienes are mediators of many pro-inflammatory and allergic reactions. Hence, the inhibition of the synthesis of leukotrienes by 15-LOX is seen as one of the therapeutic strategies in the management of inflammatory condition

[22]. In this study, the ethanol root extract of *N. imperialis* exhibited a higher ability to stabilize lipoxygenase activity than diclofenac, indicating that the ethanol root extract of *N. imperialis* will decrease lipoxidase activity.

During neurotransmission, acetyl cholinesterase is released from the presynaptic neuron into the synaptic cleft and binds to acetyl cholinesterase receptors on the post synaptic membrane, relaying the signal from the nerve [23]. Acetyl cholinesterase, also located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing acetylcholine. The liberated choline is taken up again by the presynaptic neuron and acetylcholine is synthesized by combining with Acetyl-CoA through the action of choline acetyl transferase [24]. Usually a cholinomimetic drug disrupts this process by acting as a cholinergic neurotransmitter that is impervious to acetyl cholinesterase's lysing action.

## 5. CONCLUSION

The present study therefore suggests that leaf extracts of *N. imperialis* has good ant-oxidative and anti-inflammatory potential and could be ameliorative in diseases due to inflammation and oxidation.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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