Vol. 9(1), pp. 1-10, October 2021
https://doi.org/10.14662/arjb2021020

ISSN: 2384-616X
https://www.academicresearchjournals.org/ARJB/Index.htm

Academic Research Journal of Biotechnology

Full Length Research

Antioxidants and Ameliorative Potentials of Stem Bark Extract of *Anogeissus leiocarpus* against Formalin - Induced Pain and Inflammation in Wistar Rats

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Submission Date: 17 September 2021 Accepted 5 October 2021

The study was carried out to reveal the toxicity dosage, antioxidant and anti-inflammatory activities of hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* (Al) in albino rats. The antioxidant ability of hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* was carried out using various antioxidant assays. The toxicity study was also conducted using 50% inhibitory concentration (IC_{50}). Formalin-induced paw oedema model was used to investigate anti-inflammatory effects of hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* in experimental rats. There is a dearth of information on the anti-inflammatory activity of this study plant in an oedema model induced by formalin and inflammatory mediators in rats. Therefore, this research was to evaluate the toxicity dosage, antioxidant and anti-inflammatory activities of hexane and ethanolic stem bark extracts of *Anogeissus leiocarpus* (Al) in wistar albino rats to validate scientifically the folklore claims of the plant. The results showed that free radicals were statistically scavenged at $p < 0.05$ by stem bark extract of the study plant at different concentrations. The higher percentage hydroxyl scavenging ability was recorded in hexane extract of stem bark of Al at 100 µg/ml (80.70 ± 0.22) while lower percentage hydroxyl scavenging ability was recorded by ethanolic extract of Al (61.19 ± 0.62). Hexane extract showed higher percentage of inhibition of DPPH activity (80.95 ± 0.86) while ethanolic extract recorded lower percentage potential of 60.26 ± 0.18 at 100 µg/ml, in a concentration dependent manner. FRAP values of ethanolic stem bark extract (46.67 ± 0.33 (mg AA/g extract)) was lower than the hexane stem bark extract (80.55 ± 1.97 (mg AA/ g extract) of *Anogeissus leiocarpus*). Daily oral administration of hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* was done for 14 days. No mortality was recorded after fourteen (14) days of treatment. The studied plant is safe and nontoxic to rats (LD_{50}> 5000 mg/kg).The highest percentage inhibition was exhibited by hexane extract at dose of 300 mg/kg body weight while the lowest percentage paw oedema inhibition (33.57 %) was observed in ethanolic extract at dose of 75 mg/kg. The standard drug (ibuprofen) at 400 mg/kg body weight exhibited the percentage of inhibition of 64.70%. The antioxidant potentials and the ability of the stem bark extract of *Anogeissus leiocarpus* to reduce the size of oedema produced by formaldehyde, suggested that it has chemical constituents that may be active against inflammatory disorders.

Key words: *Anogeissus leiocarpus*, *antioxidant, formalin, anti-inflammatory.*

Cite This Article As: Akande, T., Salawu, S.O., Akindahunsi, A.A., Mulinacci, N. ((2021). Antioxidants and Ameliorative Potentials of Stem Bark Extract of *Anogeissus leiocarpus* Against Formalin - Induced Pain and Inflammation in Wistar Rats.. Acad. Res. J. Biotech. 9(1): 1-10

INTRODUCTION

The induction of pain by formalin is due to the inflammation of the peripheral tissue, leading to a central sensitization of dorsal horn neurons during the inflammatory pain (Ji *et al*., 2018). Formalin-induced pain is characterized by exudation of fluid and plasma proteins and the emigration of leukocytes, majorly neutrophils (Jang *et al*., 2020). Several works have reported that formalin injection also produces an oedema and an increase of vascular permeability in the injected paw. The first response of the immune system to infection or irritation that helps the body to protect itself against an injurious stimulus is inflammation (Jang *et al*., 2020). Inflammation can be brought about by physical injury, antibodies or infections (Chen *et al*., 2017). There are two types of inflammation (acute or chronic) depending on the nature of the effectiveness of the initial reaction or the stimulus in eliminating the stimulus or the damaged tissues (Ashmawi *et al*., 2016; Asahara *et al*., 2015).

Nonsteroidal Anti-inflammatory drugs are currently used for management of pain and inflammatory disorders. These drugs have adverse side effects including gastrointestinal bleeding and peptic ulcers. Therefore, there is need to explore other sources especially of plant origin for new production of anti-inflammatory drugs that are readily available, more effective and less toxic than the synthetic drugs. African birch (*Anogeissus leiocarpus* DC. Guill & Perr.) belongs to Combretaceae, includes eight species that are distributed in Asia and Africa. *Anogeissus leiocarpus* has also been reported as a good source of traditional medicine for wound healing, skin diseases and microbial infection (Dzeufiet *et al*., 2014).

Therefore, this study seeks to carry out the antioxidant and anti-inflammatory potentials of stem bark extract of *Anogeissus leoicarpus* against formalin - induced pain and inflammation in wistar rats, to validate the folklore use of this study plant on experimental inflammation in rats.

MATERIALS AND METHODS

Plant Collection and Extraction

One (1) kg of stem bark of *Anogeissus leiocarpus* was purchased from herbal practitioners at Akure main market, Akure, Ondo State, Nigeria. Identification and authentication of the study plant was carried out by the Crop, Soil and Pest Management (CSP) Department at the Federal University of Technology, Akure (FUTA), Nigeria. Voucher sample was deposited at the institution herbarium with reference number FUTA/HB/0259 allotted. Also, the voucher specimen was deposited in the Department of Neurofarba, Pharmaceutical and Neuroceutical section, Research Unit of Phytolab, University of Florence, Italy. The air-dried stem bark was coarsely powdered and subjected to cold extraction using hexane and 70% ethanol solvents separately for 72 hours, the filtrate was concentrated using rotary evaporator and freeze dried to obtain crude extract.

Drugs and Chemicals

Ibuprofen (Ranbaxy), formalin and other chemicals were of analytical grade.

Evaluation of Antioxidant Components and Activity

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay

The ability of *Anogeissus leiocarpus* (Al) to scavenge the DPPH radicals was assessed by using the method described by Molyneux (2004) with a slight modification. DPPH solution (0.1 mmol/l) was prepared in methanol and 0.5 ml was added to different concentrations of Al (25, 50 75 and 100 µg/ml). The mixture was incubated at room temperature for 30 min. A control reference was carried out which contains methanol as a baseline correction instead of Al. After incubation, absorbance was taken at 517 nm. The result was compared with the ascorbic acid, a standard antioxidant. Percentage DPPH radical scavenging activity was calculated and plotted against concentration. IC_{50} value (the microgram of extract to scavenge 50% of the radicals) was evaluated using linear regression analysis.

% DPPH inhibition = $\frac{(Abs\ of\ Control - Ab\ \ of\ Sample)}{(Abs\ of\ Control)} \times 100$

Absorbance of control (without sample) = Abs control; Absorbance of Al= Abs sample.

ABTS radical cation decolorization assay

Free radical scavenging ability of the Al extract was determined by ABTS radical cation decolorization assay and was carried out according to the method of Re et al. (1999). ABTS·+ cation radical was produced by reacting 7 mM of ABTS and 2.45 mM potassium persulfate (1:1) and stored in the dark at room temperature for 12-16 h before use. ABTS·+ solution was then diluted with 80% methanol to obtain absorbance between 0.700 at 734 nm. Then, 100 µl of different concentration of Al or Trolox (25, 50, 75 and 100µg/ml) was added to 2 ml of diluted ABTS·+ solution. The mixture was incubated for 10 min in the dark and absorbance was taken at 734 nm. A control and solvent blank were run for the assay. The entire assay was done in triplicate. Trolox was used as reference standard. IC_{50} value (the microgram of extract to scavenge 50% of the radicals) was evaluated using linear regression analysis

Percentage ABTS cation radical inhibition was calculated from the formula,

% ABTS cation radical inhibition =$\frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control} \times 100$

Absorbance of control= Abs control, Absorbance of Al = Abs sample.

Hydroxyl Radical Scavenging Activity

This assay was described by Jin *et al.,* (1996).The hydroxyl radical was generated in a mixture of 1.0 ml of 0.75 mM 1,10-phenanthroline, 2.0 ml of 0.2 M sodium phosphate buffer (pH 7.4), 1.0 ml of 0.75 mM $FeSO_4$ and 1.0 ml of H_2O_2 (0.01%, v/v). After addition of 1.0 ml extract at various concentrations (25, 50, 75 and 100 µg/ml), the mixture was incubated at 37°C for 30 min. Then, the absorbance of the mixture at 536 nm was measured. Ascorbic acid also at different concentrations (25, 50, 75 and 100 µg/ml) was used as reference standard and was treated the same way as that of extracts. Distilled water was used as the blank. The scavenging activity on -OH was calculated by the following equation:

Scavenging activity (%) = {(Abs sample – Abs blank)/ (Abs0 – Abs blank)} *100
Where, Abs 0 is the absorbance of the distilled water instead of H_2O_2 and extract in the assay system

Nitric Oxide Radical Scavenging Activity

This assayed was described by Sangameswaran *et al.,* (2009). Various concentrations (25, 50, 75 and 100 µg/ml) of the extract and standard were prepared. Sodium nitroprusside (2.5 ml, 10 mM) in phosphate buffered saline (PBS) was added to 0.5ml different concentrations of extract and standard. The reaction mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml aliquot was removed and 0.5 ml of Griess reagent: (1% (w/v) added. The absorbance was measured at 546 nm. Ascorbic acid was used as reference standard and was treated the same way as that of extracts. Sodium nitroprusside in PBS (2 ml) was used as control. The nitric oxide radicals scavenging activity of the extracts and ascorbic acid was calculated according to the following equation:
Percentage of inhibition = [(Ao– A1) / Ao] x 100.

Where Ao is the absorbance of sodium nitropruside in PBS (without extract or ascorbic acid) and A1 is the absorbance in the presence of the extract or ascorbic acid.

Ferric Reducing-Antioxidant Property (FRAP) Assay

The reducing property of the sample was determined by method described by Pulido et al. (2000).FRAP reagent solution containing 300 mM acetate buffer, 10 mM TPTZ in 40 mMHCl, 20 mM $FeCl_3.6H_2O$ (10:1:1 respectively) was prepared. Three millilitre of prepared FRAP reagent was mixed with 100 µl *Anogeissus leiocarpus* (Al) extract (1 mg/ml) and the absorbance at 593 nm was recorded after a 30 min incubation at 37°C. A linear graph of $FeSO_4$ reference standard was prepared and the FRAP value of Al was extrapolated from it. FRAP value was expressed as mMol Fe^{2+} equivalents per g dry weight.

Determination of Total Phenolics Content

The total phenolic content of extracts was determined using the Folin-Ciocalteu method as modified by Hung *et al*. (2001). Extracts (Al) (0.1 ml) was rapidly mixed with 0.1 ml of Folin-Ciocalteu reagent, followed by the addition of 0.3 ml sodium carbonate (15%, w/v) solution. The mixture was incubated in the dark for 30 mins. The absorbance of the blue colour was read at 760 nm after 30 mins on a spectrophotometer. The total phenolic content was extrapolated from a standard curve using gallic acid (graded doses, 25 – 100 ug/ml) as a standard phenolic acid. The amount of total phenolics was expressed as gallic acid equivalents (GAE, mg gallic acid/g Al).

Determination of Total Flavonoid Content

The total flavonoid content was determined spectrophotometrically according to the modified method of Kumaran and Karunakaran (2007). Briefly, 0.5 ml of Al solution and standard (quercetin) at different concentrations (25 - 100µg/ml) were taken in test tubes dissolved in methanol followed by the addition of 0.1 ml of 10% aluminium chloride solution. 0.1 ml of 1M sodium acetate solution was added to the mixtures in the test tubes. Furthermore, each reaction test tube was then immediately diluted with 2.8 ml of distilled water and mixed to incubate for 30 min at room temperature to complete reaction. The absorbance of pink coloured solution was noted at 415 nm using a spectrophotometer against blank methanol. The total flavonoids content was expressed as mg/g Quercetin Equivalent (mg/g QE).

Animals

Albino wistar rats weighing approximately (160–250 g body weight) were used. Rats were housed and used at least one week after their arrival in plastic cages with filter tops at the Animal House Lab, Department of Biochemistry, Federal University of Technology Akure, Ondo State, Nigeria. Six rats were housed per cage; animals were fed a standard laboratory diet and tap water ad libitum and kept at under standard environmental conditions (23–25°C, 12-h light/12-h dark cycle) (OECD,2010).

Acute Toxicity Test

Rats `were` orally administered hexane and ethanolic stem bark extract of *Anogeissus leiocarpus albidum* singly at varying dosage (0-5000 mg/kg b.w.) on day 1 and the animals were observed for 24 hours to detect any sign of toxicity or death as well as delayed toxic symptoms for 7 days (Lorke,1983).

Induction of inflammation using rat model of formalin-induced paw oedema (Experimental Protocol)

Anti-inflammatory activity of hexane and ethanolic stem bark of *Anogeissus leiocarpus* was measured in rat model of formalin-induced paw oedema. Albino rats fasted overnight were divided into 9 groups of six animals each, the dosage of the drugs administered to the different groups was as follows.

Group I- control (normal saline)
Group II- Formalin induced (0.1ml/kg bw)
Group III-V -Formalin + (hexane extract of Al; 75, 150 and 300 mg/kg b.w) for 14 days
Group VI-VIII -Formalin + (ethanol) extract of Al.; 75,150 and 300 mg/kg b.w) for 14 days
Group IX - Standard drug (Ibuprofen) for 14 days

Formalin-induced paw oedema, pain and inflammation

Thirty minutes pre oral treatment with extract/drug, following injection of formalin (0.1ml of 10% v/v) into the right hind paw of the tested rats. No injection of formalin into the control group animals. The paw thickness was measured before and after induction of inflammation by using vernier calliper. The increase in paw oedema was measured by vernier calliper according to method described by Taylor *et al*. (2000) and Joseph *et al.* (2005) with some modifications.

The difference in paw thickness after and before induction of inflammation was calculated and presented as mean increase in paw thickness (cm). The ability of hexane and ethanolic stem bark extract of *Anogeissus leoicarpus* and the standard (ibuprofen) used as anti-inflammatory drugs to suppress paw inflammation was expressed as a percentage of inhibition of paw oedema (Taylor *et al*., 2000; Joseph *et al.,* 2005).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel and Graph Pad prism 8 software. Data were analyzed using student t-test and analysis of variance (One-way ANOVA) followed by Turkey's test. Values of $p < 0.05$ were considered as statistically significant.

RESULTS

Table 1. DPPH (%) Scavenging Ability of Hexane and Ethanolic Stem Bark Extract of *Anogeissus leoicarpus* (Al)

Concentration (µg/ml)	Hexane	EtOH	Ascorbic acid
25	32.99 ± 0.18^{h}	21.56 ± 0.37^{l}	50.48 ± 0.80^{f}
50	50.91 ± 0.18^{f}	37.84 ± 0.31^{g}	64.42 ± 2.20^{d}
75	60.69 ± 0.49^{e}	48.83 ± 0.37^{f}	75.15 ± 1.53^{c}
100	80.95 ± 0.86^{b}	60.26 ± 0.18^{e}	91.43 ± 0.18^{a}

Values (means of three replicates) followed by different letters are significantly different at $p < 0.05$

Table 2. Hydroxyl Radical (%) Scavenging Ability of Hexane and Ethanolic Stem Bark Extract of *Anogeissus leoicarpus* (Al)

Conc (µg/ml)	Hexane	EtOH	(Ascorbicacid)
25	34.29 ± 5.44^{k}	21.46 ± 0.87^{l}	60.37 ± 1.31^{g}
50	$62.52 \pm 0.44^{f, g}$	44.76 ± 4.38^{j}	71.56 ± 3.70^{d}
75	$70.12 \pm 0.22^{d, e}$	$53.39 \pm 1.24^{h, i}$	85.83 ± 0.87^{b}
100	80.70 ± 0.22^{c}	61.19 ± 0.62^{g}	94.35 ± 0.22^{a}

Values (means of three replicates) followed by different letters are significantly different at $p < 0.05$.

Table 3. Nitric Oxide (%) Scavenging Ability of Hexane and Ethanolic Stem Bark Extract of *Anogeissus leoicarpus* (Al)

Conc (µg/ml)	Hexane	EtOH	(Ascorbic acid)
25	35.68 ± 0.18$^{l, m}$	21.08 ± 8.74^{n}	63.06 ± 0.18$^{e, f}$
50	53.93 ± 0.38^{i}	38.32 ± 0.63$^{k, l}$	78.56 ± 1.08^{d}
75	62.70 ± 0.79$^{e, f, g}$	42.82 ± 0.63^{j}	89.79 ± 0.45^{b}
100	82.64 ± 0.55^{c}	58.92 ± 0.18^{h}	97.42 ± 0.38^{a}

Values (means of three replicates) followed by different letters are significantly different at $p < 0.05$

Table 4. ABTS Assay (%) of Hexane and Ethanolic Stem Bark Extract of *Anogeissus leoicarpus* (Al)

Conc (µg/ml)	Hexane	EtOH	(Trolox)
25	63.20 ± 0.74^{f}	36.33 ± 0.58^{j}	71.07 ± 0.28^{d}
50	67.23 ± 0.58^{e}	41.57 ± 0.56^{i}	77.99 ± 0.86^{c}
75	78.84 ± 0.43^{c}	45.88 ± 0.43^{h}	84.46 ± 0.71^{b}
100	85.21 ± 0.86^{b}	53.37 ± 0.74^{g}	95.88 ± 0.32^{a}

Values (means of three replicates) followed by different letters are significantly different at $p < 0.05$

Table 5. IC_{50} of *In vitro* Assay of Hexane and Ethanolic Stem Bark Extract of *Anogeissus leiocarpus* (Al)

***In vitro* assay (%) IC_{50} (µg/ ml)**	**Hexane**	**Ethanol**	**Standard**
DPPH Scavenging Ability	55.17 ± 2.18	80.10 ± 6.32	55.29 ± 4.86
OH Scavenging Ability	47.50 ± 1.33	47.94 ± 4.29	58.21 ± 3.59
NO Scavenging Ability	56.70 ± 0.99	65.56 ± 3.92	100.13 ± 6.29
ABTS Scavenging Ability	48.11 ± 6.84	46.23 ± 5.53	41.38 ± 6.39

Values (means of three replicates) followed by different letters are significantly different at $p < 0.05$

Table 6. Phenolic Content and Antioxidant Activities of Hexane and Ethanolic Stem Bark Extract of *Anogeissus leoicarpus*

Phytochemicals	Hexane	Ethanol
Total Phenolic Content	69.84 ± 0.16 (mg GAE/ g extract)	53.28 ± 0.15 (mg GAE/ g extract)
Total Flavonoids Content	60.75 ± 0.31 (mg QUE/ g extract)	50.34 ± 0.42 (mg QUE/ g extract)
Total Antioxidant Capacity	70.00 ± 2.15 (mg AAE/g extract)	58.70 ± 0.31(mg AAE/g extract)
FRAP	80.55 ± 1.97 (mg AA/g extract)	46.67 ± 0.33(mg AA/g extract)

Key: AA -Ascorbic acid; GAE- Gallic acid; QUE- Quercetin; FRAP- Ferric reducing-antioxidant property

Table 7. Anti-inflammatory Potential of Hexane and Ethanolic Stem bark Extract of *Anogeissus leiocarpus* against Formalin -Induced Paw Oedema/ Inflammation in Rats

	0 min	30mins	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	% Inhibition in paw oedema
Control	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	100%
Induced	0.33 ± 0.03^a	0.60 ± 0.04^a	0.65 ± 0.03^a	0.74 ± 0.09^a	0.89 ± 0.01^a	0.86 ± 0.02^a	0.84 ± 0.02^a	0.81 ± 0.05^a	-
I + Al Hex (75 mg/kg)	0.35 ± 0.04^a	0.42 ± 0.03^a	0.49 ± 0.04^a	0.58 ± 0.04^a	0.72 ± 0.02^a	0.62 ± 0.01^a	0.61 ± 0.00^a	0.61 ± 0.00^a	45.45%
I + Al Hex (150 mg/kg)	0.36 ± 0.01^a	0.60 ± 0.02^a	0.62 ± 0.02^a	0.65 ± 0.03^a	0.70 ± 0.02^a	0.61 ± 0.02^a	0.60 ± 0.03^a	0.60 ± 0.03^a	50.70%
I + Al Hex (300 mg/kg)	0.36 ± 0.02^a	0.44 ± 0.04^a	0.48 ± 0.03^a	0.55 ± 0.03^a	0.64 ± 0.04^a	0.58 ± 0.06^a	0.57 ± 0.06 ^{a}	0.57 ± 0.06^a	56.29%
I + Al EtOH (75 mg/kg)	0.36 ± 0.02^a	0.46 ± 0.03^a	0.53 ± 0.0^a	0.62 ± 0.04^a	0.72 ± 0.05^a	0.69 ± 0.05^a	0.68 ± 0.04^a	0.67 ± 0.05^a	33.57%
I + Al EtOH (150 mg/kg)	0.35 ± 0.02^a	0.47 ± 0.02^a	0.52 ± 0.04^a	0.61 ± 0.06^a	0.74 ± 0.05^a	0.66 ± 0.07^a	0.66 ± 0.06^a	0.64 ± 0.07^a	39.16%
I + Al EtOH (300 mg/kg)	0.35 ± 0.03^a	0.57 ± 0.04^a	0.61 ± 0.01^a	0.64 ± 0.02^a	0.69 ± 0.06^a	0.65 ± 0.04^a	0.64 ± 0.04^a	0.62 ± 0.04^a	42.67%
I + Ibuprofen (400mg/kg)	0.38 ± 0.02^a	0.47 ± 0.02^a	0.55 ± 0.02^a	0.64 ± 0.03^a	0.73 ± 0.02^a	0.65 ± 0.02^a `	0.62 ± 0.02^a	0.55 ± 0.01^a	64.70%

Values (means of six replicates) followed by different letters are significantly different at $p < 0.05$

DISCUSSION

The present study revealed the antioxidant (*in vitro*) and anti-inflammatory activities of hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* (Al) in wistar albino rats. Free radicals can react with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, causing cellular damage. To find out the antioxidant ability of hexane and ethanolic stem bark extract of *Anogeissusl leiocarpus* various antioxidant assays were carried out.

DPPH radical scavenging activity is one of the most important assessments of antioxidant potency of pure compounds or crude plant extract (Saira *et al*., 2020). In DPPH radical scavenging assay, the hexane extract showed excellent activity, the hexane extract showed higher percentage potential (80.95 ± 0.86^{b}) at 100 µg/ml; IC_{50} value was 55.17 ± 2.18 µg/ml, while ethanolic extract has lower percentage potential of 60.26 ± 0.18^{e} at 100 µg/ml and IC_{50} value of 80.10 ± 6.32 µg/ml as shown in Tables 1and 6, all in aconcentration dependent manner.

In this study, both hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* (Al) quenched hydroxyl radicals and halted 2- deoxyribose breakdowns at higher concentration of 100 µg/ml (Table 2). The higher percentage hydroxyl scavenging ability was recorded in hexane extract of stem bark of Al at 100 µg/ml (80.70 ± 0.22^{c}); IC_{50} value was 47.50 ± 1.33 µg/ml followed by hexane extract at 75 µg/ml(70.12 ± 0.22$^{d, e}$); while lower percentage hydroxyl scavenging ability was recorded by ethanolic extract of Al (61.19 ± 0.62^{g}); IC_{50} value was 47.94 ± 4.29 µg/ml. It should be noted that both hexane and ethanolic extract of Al have similar IC_{50} values as shown in Table 6. The research study showed that a concentration dependent manner for hydroxyl radical quenching activity is noticed (Table 2).

In this study, the lower value of IC_{50} for nitric oxide scavenging ability is recorded by hexane extract of Al (56.70 ± 0.99µg/ml) with higher percentage scavenging activity at 100 µg/ml(82.64 ± 0.55^{c}) while IC_{50} value for ethanolic is (65.56 ± 31.92 µg/ml) at lower percentage scavenging activity at 100 µg/ml (58.92 ± 0.18^{h}) as revealed in Tables 3 and 6, in dose dependent manner.

Both the hexane (48.11 ± 6.84) and ethanolic (46.23 ± 5.53) extracts showed appreciable IC_{50} values that compared well with Trolox (41.38 ± 6.39) as shown in Table 5. At 100µg/ml, the ABTS radical scavenging capacities of hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* were 85.21% and 53.37% respectively (Table 4). The extracts demonstrated a concentration dependent ABTS scavenging capacity.

Numerous studies suggested that phenolic compounds are responsible for effective free radical scavenging and antioxidant activities (Sanjay *et al*., 2020). Therefore, determination of the phenolic content is very important in order to determine the antioxidant capacity of plant extracts (Sanjay *et al*., 2020). In the present study, higher concentration of total phenolic content was found in hexane stem bark extract of Al (69.84 ± 0.16 (mg GAE/ g extract)) while ethanolic stem bark extract recorded lower concentration of total phenolic content (53.28 ± 0.15 (mg GAE/ g extract) though suggesting the importance of stem bark extract of *Anogeissus leiocarpus* for effective antioxidant activities in traditional medicine.

Also, it was already reported that naturally occurring flavonoids compounds have free radical scavenging properties, due to their hydroxyl groups, which are effective hydrogen donors, which make them antioxidant. Higher total flavonoid content (60.75 ± 0.31 (mg QUE/ g extract)) as well as total antioxidant capacity (70.00 ± 2.15 (mg AAE/g extract) were also recorded by in hexane stem bark extract of Al while ethanolic stem bark extract recorded lower concentrations of total flavonoid content (50.34 ± 0.42mg QUE/ g extract)) and total antioxidant capacity (58.70 ± 0.31 (mg AAE/ g extract).

The Ferric reducing-antioxidant property(FRAP) values of ethanolic stem bark extract (46.67 ± 0.33 (mg AA/g extract)) was lower than the hexane stem bark extract (80.55 ± 1.97 (mg AA/ g extract) of *Anogeissus leiocarpus* as seen in Table 6.This is indicative that the stem bark extracts of the studied plant act as reductone that reduced the ferric tripyridyltriazine [Fe (III)-TPTZ] complex to ferrous tripyridyltriazine [Fe (II)-TPTZ]. This was similar to the previous study reported which revealed that, the higher the FRAP value the higher the reducing power of the extract on electron transfer ability towards the FRAP reagents (Amaeze *et al.,* 2011).

The toxicity study which is fundamental for an adaptation of the traditional medicine was conducted to identify the tolerance limit of the study plant. According to Lorke's method, the studied plant is safe and nontoxic to rats. (LD_{50}> 5000 mg/kg).Daily oral administration of varying doses

(75,150,300 mg/kg bw) of hexane and ethanolic stem bark extract of *Anogeissus leoicarpus* was done for 14 days.No mortality was recorded after fourteen (14) days of treatment. The control and extract treated groups of animals were normal and did not show any symptoms associated with toxicity. Kabare *et al*. (2010) reported aqueous decoction of *A. leiocarpus* leaves to be between 500 to 5000mg /kg body weight. This report confirmed the low toxicity obtained in the study of hexane and ethanolic stem bark of *A. leiocarpus.*

Formalin-induced experimental models produce a distinct biphasic nociception in the paw, a first phase (lasting the first 5 mins) corresponding to acute neurogenic pain, and a second phase (lasting from 15 to 30 mins) corresponding to inflammatory pain responses as stated by Huang *et al*. (2011).In the present study, notable oedema and inflammation was observed in the experimental animals. Oedema formation in the paw is as result of a synergism between various inflammatory mediators that increased vascular permeability and blood flow as reported by John and Shobana (2012).However, pretreatment and daily oral administration with hexane and ethanolic stem bark extract *Anogeissus leiocarpus.* showed a significant inhibition in the late phase of formalin induced pain in a dose dependent manner which was well comparable with the standard drug ibuprofen and also corroborates with the findings of Young *et al*. (2005).

The results of this study revealed that stem bark extract of as well as the standard drug (ibuprofen) significantly reduced the rat right hind paw oedema induced by subcutaneous injection of formalin, suggesting marked anti-inflammatory potentials of the studied plant in dose-dependent manner.

In conclusion, the presence of phytochemicals in the study plant can explain the anti-inflammatory activity of this study plant. Hexane and ethanolic stem bark extract of *Anogeissus leiocarpus* have revealed the significant antioxidant and anti-inflammatory potentials, thereby establishing the pharmacological basis for its use in the treatment of arthritis, inflammation, oedema and some diseases associated with oxidative stress in Nigeria ethno medicine.

Author Contributions: All authors made a substantial contribution to the analysis and interpretation of the data and to the writing and revising of the manuscript.

Conflict of interest: The authors declare that there are no conflicts of interest.

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