A STUDY OF ANTI-INFLAMMATORY ACTIVITY OF *JAUNDEA PINNATA* (P. BEAUV.) G. SCHELLENB. (CONNARACEAE) STEM BARK METHANOL EXTRACT AND FRACTIONS

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ABSTRACT

Introduction: *Jaundea pinnata* (P. Beauv.) G. Schellenb. (Connaraceae) is known for its limited medicinal uses including treatment of rheumatoid arthritis and as a repellent.

Methods: The anti-inflammatory effect of crude methanol (MeOH) extract of stem bark and aqueous (AQ) fraction was investigated by both bovine serum albumin and fresh hen's egg albumin *in vitro* models. In addition, rats dosed with MeOH extract (250 - 1000 mg/kg), and standard anti-inflammatory drug, Diclofenac sodium® (50 mg/kg) up to 14 days were investigated for effects on rat paw oedema in a formaldehyde model, as well as alteration of haematological and biochemical parameters.

Results: Oral administration with methanol extract up to Day 14 produced dose-dependent inhibition in rat paw oedema diameter comparable to the standard drug. Extract also gave non-dose dependent inhibitions of erythrocyte sedimentation rate (ESR) which was higher than the standard drug. Effect of extract on haematological parameters and liver function parameters (total protein, albumin and alanine aminotransferase) was not dose-dependent, but comparable to the Diclofenac sodium® at 50 mg/kg.

Conclusion: The results of this study justify the ethnomedicinal relevance of *J. pinnata* leaf in treating rheumatoid arthritis.

Keywords: Anti-inflammatory activity, Connaraceae, *Jaundea pinnata, in vitro, in vivo,* stem bark

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, and systemic autoimmune disease with characteristic symptoms of pain, swelling, and destruction of cartilage and bone[1]. An estimate of 0.3-1% of the global population are victims of rheumatoid arthritis with the females being more susceptible than males[1]. Demerits of high costs and side effects such as high risks of infections often accompany orthodox treatment strategy involving use of disease modifying anti-rheumatic drugs (DMARDs) or non-steroidal anti-inflammatory drugs (NSAIDs)[1,2]. Current treatment approach through the use of medicinal plants is advantageous being more acceptable, relatively cheaper, safer, and polyherbal formulations are prepared to reduce side effects and increase the benefits[1]. In Nigeria, prevalence studies of RA have been documented in the north east[3], Rivers state[4] and Lagos[2], and preponderance of the female population to the disease were reported.

Furthermore, the increasing prevalence of RA in Nigeria[1] has resulted in the emergence of the country as a leading nation in anti-inflammatory research among developing nations globally.

Jaundea pinnata (P. Beauv.) G. Schellenb (Connaraceae) is a shrub or small tree attaining 6-7m and widely spread from Guinea to South Nigeria, to North East and East Africa[5]. Fruit is poisonous to animals in Kenya, and stem a repellent. The plant is employed in folkloric treatment of rheumatoid arthritis is south west Nigeria (personal communication). Limited published work on *J. pinnata* included the antibacterial activity against plasmid-bearing multiple antibiotics resistant bacteria[6] and recently, anti-inflammatory potential of its leaf[7]. Ethnobotanical surveys of Nigerian anti-rheumatic plants in Lagos[8], Ogun state[9], the South West[10] and North West[11] have been published without mention of *J. pinnata*.

Global reviews of anti-inflammatory activity of medicinal plants in India[12,13] and elsewhere[1, 14] is a pointer to increasing scientific research in this subject area. Furthermore, some Nigerian plants[14,15,16] and others growing elsewhere[17,18,19] have been investigated for anti-inflammatory activities. The recent publication on anti-inflammatory activity of *J. pinnata* leaf[7], and the growing interest at discovering promising anti-inflammatory medicinal plants have stimulated investigation of *J. pinnata* stem bark by *in vivo* and *in vitro* experimental models, and results are published herein.

MATERIALS AND METHODS

Chemicals and reagents

Formaldehyde (Needham Market, Suffolk, England), standard drug, Diclofenac sodium®, sodium chloride and bovine serum albumin (Sigma-Aldrich, USA), disodium hydrogen phosphate (Merck, Germany), potassium dihydrogen phosphate (Riedel-de-Haen, USA), analytical grade chemicals (Merck), analar grade organic solvents (Sigma, UK), hydrochloric acid (BDH, England) and egg albumin from fresh hen's egg were used.

Collection, preparation and extraction of plant material

Fresh stem bark peels of *J. pinnata* were collected from trees growing at the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Ibadan in December 2019, and authenticated (voucher no. FHI 109517) by Mr. Odewo of the Institute. Plant material was cut into small pieces, shade dried at room temperature, ground into coarse powder using locally fabricated machine, and stored in an air tight container at ambient temperature. Ground stem bark (1100 g) was extracted to exhaustion with methanol (MeOH) in a Soxhlet apparatus. Crude MeOH extract (7.63%) (Table 1) was concentrated *in vacuo,* weighed and fractionated with dichloromethane (DCM) to yield DCM and aqueous fractions. Fractions were concentrated to dryness on steam water bath, yield determined (DCM 2.32%, AQ 26.85%) and refrigerated until needed.

Phytochemical screening

Basic phytochemical screening was carried out on the crude methanol extract of the plant according to Evans[20] and the presence of secondary metabolites recorded.

Inhibition of protein denaturation using bovine serum albumin (BSA) model

Evaluation of *in vitro* anti-inflammatory effects of the crude methanol extract and fractions followed the protocol described by Alamgeer *et al*.[18] with some modifications. The reaction mixture consisted of 1 mL of extract (aqueous and DCM fractions) at 100, 200, 500, and 1000 µg/mL) homogenized with 1mL aqueous solution of bovine serum albumin (5% BSA). Solution was adjusted to pH 6.3 by 1N HCL and incubated at 37°C for 15 minutes. The procedure was repeated with Diclofenac sodium® and distilled water which served as positive and negative controls, respectively. Each experiment was replicated thrice. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70°C. Mixture was cooled at the ambient room temperature, then 0.5 mL of phosphate buffer saline was added and the absorbance measured spectrophotometrically (UV/VIS Spectrophotometer UV 752 (D) PEC Medical, USA) at 660nm. Percentage inhibition was calculated from the formula:

Absorbance control - Absorbance test x 100

Absorbance control

Inhibition of protein denaturation in a fresh hen's egg albumin (FHEA) model

Modified procedure of Alamgeer *et al*.[18] was employed to evaluate the *in vitro* anti-inflammatory effects of the extract and fractions. Reaction mixture (5 mL) consisted of 0.2 mL fresh hen's egg albumin, 2.8 mL phosphate buffered saline (pH 6.4), and 2 mL of different concentrations (100, 200, 500 and 1000 µg/mL) of each test agent. Diclofenac sodium® dissolved in 2 mL double distilled water served as positive control. Mixtures were incubated at 37°C for 15 min and heated on a water bath at 70°C for 5mins. Absorbance was measured spectrophotometrically (UV/VIS Spectrophotometer UV 752 (D) PEC Medical, USA) at 660nm and percentage inhibition calculated from the formula:

$$\frac{\text{Absorbance control} - \text{Absorbance test}}{\text{Absorbance control}} \times 100$$

Experimental Animals

Male Wistar rats weighing 180–200g were obtained from the central animal house of Igbinedion University, Okada, Nigeria. They were housed in 12 h light & dark cycle condition (28-35°C) and fed with pelleted chew (Bendel Foods and Flower Meal, Edo state, Nigeria) and water *ad libitum* for 2 weeks. The animals were handled in compliance with the Igbinedion University Okada Ethics Committee on Research in Animals, as well as internationally accepted principles for laboratory animal use and care.

Evaluation of formaldehyde-induced inflammation

The method of Alamgeer *et al*.[18] was adopted in this experiment. Animals were divided into five groups of six animals each. Oedema was induced by injection of 0.1 mL of 1% formaldehyde in normal saline, subcutaneously in the plantar side of the right hind paw of each rat in all groups. Inflammation was assessed by measurement of paw diameter with manual Vernier caliper before formaldehyde injection was given. Induction of oedema was repeated for three consecutive days (Day 1 – Day 3) and diameter of inflammation measured again on the third day of induction. At the end of this period (representing Day 1), groups A and B were orally administered with distilled water (10 mL/kg) and standard anti-inflammatory drug, Diclofenac sodium® (50 mg/kg) respectively for 14 days, while animals in groups C, D and E received 250, 500 and 1000 mg/kg extract for same period, respectively. Diameter of inflammation was measured on Days 1, 7 and 14 to determine anti-inflammatory effect. On Day 14, animals were anaesthetized in a jar containing diethyl-soaked cotton wool, and blood collected by cardiac puncture into heparinized tubes for haematological, erythrocyte sedimentation rate (ESR) and liver and renal enzymes assays.

Percentage inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = (Dc - Dt)/ Dc \times 100$$

Where Dc is the average inflammation (hind paw edema) of the negative control group of rats at a given time; and Dt is the average inflammation of the drug- treated (i.e. extract or reference drug) rats at the same time.

Determination of serum liver and renal functions

Treated rats from the formaldehyde experiment were sacrificed on Day 14 by cervical decapitation after mild diethyl ether anaesthesia, a ventral longitudinal abdominal incision made and the liver and kidney were identified and immediately dissected out as described by Adeneye *et al*[21]. These dissected organs were separately rinsed and homogenized in ice-cold 0.01M Tris HCL buffer, pH 7.4 to give a 10% homogenate, and used to estimate liver and renal function parameters. Serum albumin (ALB), total protein (TP), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and globulin (GLOB) as liver function parameters, and serum urea and creatinine (CRT) as renal function parameters, were assayed for using Roche and Cobas commercial test kits and Roche/Hitachi 904 Automated Analyzer.

Haematological analysis

The full hematological parameters evaluated on Day 14 after the formaldehyde experiment according to Adeneye *et al*[21] include haemoglobin (Hb), white blood cell (WBC) count, neutrophil and packed cell volume (PCV) were assayed using Roche and Cobas commercial test kits and Roche/Hitachi 904 Automated Analyzer.

Estimation of erythrocyte sedimentation rate

Erythrocyte sedimentation rate (ESR) was assessed on Day 14 after the formaldehyde experiment according to Chitme and Patel[22] in Westergren pipettes having 2.5 mm internal diameter, 300 mm length, and 1 mL capacity and ESR stands. Blood was collected from all the arthritic and non-arthritic animals used in the study by cardiac puncture. Percentage (%) inhibition was calculated from the formula:

$= (Dc - Dt)/ Dc \times 100$, where Dc is the average ESR count in the negative control group of rats at a given time; and Dt is the average count in the treated group (i.e. extract, fractions or standard drug) of rats at the same time.

Statistical analysis

Results of triplicate determinations were expressed as mean±SEM and subjected to statistical analysis using one-way analysis of variance (ANOVA, Krusta-Wallis test), and difference at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins and steroids in the plant (Table 2). Both MeOH extract and aqueous (AQ) fraction when tested at 100-1000 µg/mL, produced concentration-dependent inhibition of heat-induced protein denaturation in bovine serum albumin (BSA) model (Table 3), but were incomparable ($p<0.05$) to standard drug, Diclofenac sodium. The fraction (34.12% inhibition) was less active than MeOH extract (57.15% inhibition) at 1000 µg/mL. In the fresh hen's egg albumin (FHEA) model, only AQ fraction was concentration-dependent up to 500 µg/mL in inhibition of protein denaturation (Table 4). Its activity was significant ($p<0.05$) and less (38.51% inhibition at 1000 µg/mL) when compared to standard drug. In considering both protein denaturation experiments, since both active AQ fraction (38.51% inhibition at 1000 µg/mL) and standard drug (96.46% inhibition) gave higher inhibitions in FHEA model, this model may appear to be a more effective tool in determining anti-inflammatory potential of *J. pinnata* stem bark. Limited amount of DCM fraction did not permit its use for various assays. This result is a reversal of the observations recently reported for *J. pinnata* leaf which showed better activity in the BSA model[7]. Result of this present study is in agreement with the finding of Shilpa *et al*[23] who observed better inhibition of protein denaturation in the FHEA model for *Hibiscus hispidissimus*. Shilpa *et al*[23] also suggested protein denaturation as one of the main causes of rheumatoid arthritis due to the production of auto antigens. Mechanism of denaturation has been attributed to alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding[18].

Table 1: Profile of *Jaundea pinnata* stem bark and yield of extract and fractions

Plant	Voucher number	Morphological part	Aspect	Location
Jaundea pinnata	FHI 109517	Stem bark	Shrub	Forestry Research Institute of Nigeria, Ibadan

Yield		
Crude methanol extract	Aqueous fraction+	Dichloromethane+
7.63%	26.85%	2.32%

+Relative to crude extract

Table 2: Phytochemical screening of crude methanol extract

Phytometabolites	Result
Alkaloids	+
Tannins	+
Anthraquinone glycosides	-
Cardiac glycosides	-
Flavonoids	+
Terpenoids	-
Steroids	+
Saponins	+

Present (+), Absent (-)

Table 3: Effect of *Jaundea pinnata* stem bark extract and fraction on protein (bovine serum albumin) denaturation

Tested agent	Inhibition (%)			
	Concentration (μg/mL)			
	100	200	500	1000
Crude MeOH extract	32.45±1.92*	34.74±3.75*	48.31±0.33	57.15±0.12
AQ fraction	14.90±3.42*	19.83±3.45*	25.90±2.81*	34.12±0.27*
Diclofenac	59.45±0.12	75.36±0.95	77.85±0.51	80.31±0.92

The values above are mean of three replicates n=3, mean ± SEM, values with superscript* indicate significant difference at p <0.05 compared to control (diclofenac), while values with no superscript indicate no significant difference when compared to control (diclofenac) at p<0.05

Table 4: Effect of *Jaundea pinnata* stem bark extract and fraction on protein (fresh hen's egg albumin) denaturation

Tested agent	Inhibition (%)			
	Concentration (μg/mL)			
	100	200	500	1000
Crude MeOH extract	39.99±2.44*	37.92±4.42*	49.35±2.21*	48.41±0.25*
AQ fraction	35.14±2.81*	38.03±0.49*	41.06±2.27*	38.51±0.28*
Diclofenac sodium®	67.36±4.22	69.44±1.39	94.86±1.25	96.46±0.55

Values are mean of three replicates (n=3), Mean ± SEM. Values with superscript* indicates significant difference at p<0.05 when compared with control (diclofenac), while values with no superscript

indicate no significant difference when compared with control drug (diclofenac) at $p<0.05$ using One-way ANOVA (Krustal Wallis test)

A comprehensive review of mechanisms of action of anti-inflammatory plants has been published[1]. Thus, prevention of protein denaturation by *J. pinnata* could lend credence to its anti-inflammatory effect. The results herein are in consonance with those reported by Akinnawo *et al*.[16] and Mbiantcha *et al*[24] for *Alstonia boonei* and *Piptadeniastrum africanum*, respectively. Furthermore, there appear to be no significant difference in protein denaturation effect of MeOH extract at all concentrations in both BSA and FHEA assays. Anti-inflammatory activity displayed by *J. pinnata* could be attributed to alkaloids, tannins, flavonoids, steroids and saponins[11,14] detected in this study. From this study, it may be necessary to subject AQ fraction to further phytochemical work to unravel the bioactive compounds that will serve as lead in the development of anti-inflammatory drugs.

In the haematological parameters, MeOH extract gave comparable PCV (45.20-47%), haemoglobin (15.06-15.68 g/dL), neutrophil (60.20-64 cells/mm^3) and lymphocyte (31-34.40 cells/mm^3) counts in a non-dose-dependent manner at tested doses (Table 5). However, these data were comparable to the standard drug. Extract gave higher WBC count than Diclofenac sodium® at all doses, but was not dose-dependent. PCV and haemoglobin counts can be considered to be near normal with reference to untreated control. Elsewhere, elevated levels of serum haemoglobin were observed to aid recovery from arthritis[19,22,24]. In this study, the near normalisation of PCV and haemoglobin parameters by MeOH extract (with reference to untreated arthritic control) and comparable levels of all indices (except WBC) with standard drug, is a pointer to anti-inflammatory potential of *J. pinnata* stem bark. These findings are consistent with those of Gbolade *et al* for *J. pinnata* leaf[7] and Adeneye *et al*[21].

Table 5: Effect of *Jaundea pinnata* stem bark extract and aqueous fraction on haematological parameters on rats

Tested agent	WBC (cells /µL)	LYMP (cells/µL)	MONO (cells /µL)	GRAN (cells /µL)	RBC (10^3 cells /µL)	HGB (g/dL)
Negative Control (distilled water, 10 mL/kg)	5200±1140	3170±670	1300±380	730±90	6730±80	14.53±0.66
Positive Control (50 mg/kg)	6000±1720	4600±2510	900±150	400±32	7290±4510	16.60±3.99
Crude MeOH extract (250 mg/kg)	6100±1200	4500±1100	1030±30	500±100	6770±330	14.40±0.80
Crude MeOH extract (500 mg/kg)	10,770±920*	9000±3420*	1600±11	1000±20	6980±1110	13.80±2.81
Crude MeOH extract (1000 mg/kg)	11,600±920*	6430±150	2330±630	2000±450	6820±140	14.67±0.48
AQ fraction (100 mg/kg)	8870±2320	4530±960	1100±100	2200±1140	6530±460	13.97±1.24
AQ fraction (200 mg/kg)	6530±930	4470±570	2130±640	930±230	6070±50	13.50±0.3

WBC, white blood cells; LYMP, lymphocytes; MONO, monocytes; GRAN, granulocytes; RBC, red

blood cells; HBG, mean corpuscular haemoglobin. Values with superscript* indicate significant difference ($p<0.05$) relative to negative control.

Inhibition of rat paw oedema increased up to Day 7 with tested doses of MeOH, and fell by Day 14 (Table 6). Extract at lower doses, 250 and 500 mg/kg, were equipotent and gave maximal inhibition (56.30%) on Day 7. However, dose-dependent inhibition of inflammation was recorded with MeOH extract only on Day 14. All tested doses of extract produced insignificantly different inhibitions on Day 7 (54.76-56.30%) and Day 14 (44.80-50.40%) which were comparable to Diclofenac sodium. Results obtained in this study indicate anti-inflammatory potential of *J. pinnata* stem bark, and are in consonance with those documented for *J. pinnata* leaf[7] after 14 days, and for *Alchornea cordifolia*[21] and *Xanthium srtumarium*[17] after 28 days. Reduction of localised inflammation and biphasic pain induced by formaldehyde by *J. pinnata* extract possibly suggests[18] central and peripheral inhibition of the arachidonic pathway. Other potent anti-inflammatory plants have been previously reported[18,19,24].

Table 6: Effect of *Jaundea pinnata* stem bark methanol extract and aqueous fraction on formaldehyde-induced inflammation

Tested agent	Degree of inflammation (mm)/ % inhibition#			
	Day 0	Day 1	Day 7	Day 14
Negative control (distilled water, 10 mL/kg)	8.30±1.20	10.67±0.47	10.67±0.47	10.60±0.79
Positive control (Diclofenac, 50 mg/kg)	8.00±0.68 (3.61)	9.87±0.41 (7.49)	9.87±0.41 (10.13)	9.17±0.43 (13.49)
Crude MeOH extract (250 mg/kg)	6.07±0.07 (26.86)	n. d	9.60±0.45 (10.02)	7.13±0.09 (32.73)
Crude MeOH extract (500 mg/kg)	4.77±0.72* (42.53)	13.07±1.13 (-22.49)	9.40±0.31 (11.90)	6.90±0.80* (34.90)
Crude MeOH extract (1000 mg/kg)	5.67±1.58* (31.68)	12.60±1.53 (-18.08)	9.27±0.12 (13.12)	5.93±1.16* (44.05)
AQ fraction (100 mg/kg)	6.27±1.30 (24.45) (0)	10.67±0.33	8.67±0.12 (18.74)	5.17±0.08* (51.22)
AQ fraction (200 mg/kg)	4.87±0.91* (41.32)	11.10 0.95 (-4.02)	7.87±0.17* (26.24)	5.10±0.10* (51.88)

Values above are mean of six replicates. n=6 (±SEM). Values with superscript * indicate significant difference at $P<0.05$ when compared to negative control using ordinary One –way ANOVA. #inhibition (%) relative to negative control, n.d = not determined.

ESR denotes haematological index for the diagnosis and prognosis of infections and inflammatory diseases[18]. From this study, crude MeOH extract at 250 - 1000 mg/kg did not give dose-dependent inhibition in ESR (39.97-42.70%) in arthritic rats, but was more active than the standard drug (21.55%) (Table 7). This is contrary to an earlier report documented for *J. pinnata* leaf[7]. Tested doses of MeOH extract gave varying responses to biochemical parameters considered in this study.

On the renal function parameters, near normalized serum levels of urea (63.36 mg/dl) and CRT (0.54 mg/dl) in extract-treated rats at 1000 mg/kg with untreated arthritic control were observed (Table 8). Extract was comparable to standard drug in serum urea level but yielded less CRT. Among the renal function indices investigated, serum ALT, ALP and TP observed in extract-treated rats (1000 mg/kg) were comparable to Diclofenac sodium, while serum GLOB was less. Moreover, dose-dependent increase in serum AST in extract-treated animals (77.30-96.18 U/I) which was higher than Diclofenac sodium was evident. Findings of some of the liver and renal function parameters investigated in this study are in agreement with an earlier report on *J. pinnata* leaf[7] and for other plants[21,23,24], and suggest potential for *J. pinnata* as an anti-inflammatory agent. Raceline *et al*.[19] have linked significant increase in the activity of liver function enzymes such as AST and ALT in this study in untreated arthritic animals to destructive effects of inflammation inducing agent on liver cells expressed by significant release of transaminases, and on kidney by renal cells necrosis that result in to glomerular filtration impairment. Therefore, *J. pinnata* stem bark may have potential as an anti-inflammatory agent since comparable activities with standard drug were observed for TP (8.36 mg/dl), urea (63.36 mg/dl), ALT (32.45 U/I) and ALP (247.49 U/I).

Table 7: Effect of *Jaundea pinnata* stem bark methanol extract administered for 14 days on erythrocyte sedimentation rate (ESR) in rats

Tested agent	Erythrocyte sedimentation rate (mm/hr)/ % inhibition#
Negative control (distilled water; 10 mL/kg)	7.33±3.84
Positive control (Diclofenac; 50 mg/kg)	5.75±0.85 (21.55%)
Crude methanol extract, 250 mg/kg	4.20±0.58* (42.70%)
Crude methanol extract, 500 mg/kg	4.40±0. 51* (39.97%)
Crude methanol extract, 1000 mg/kg	4.20±0.37* (42.70%)

Values above are mean of six replicates. n=6 (±SEM). Values with superscript * indicate significant difference at $P<0.05$ when compared to negative control using ordinary One –way ANOVA, #inhibition (%) relative to negative control.

Table 8: Effect of *Jaundea pinnata* stem bark methanol extract on some biochemical parameters

Parameters	Control		Crude methanol extract		
	Negative control (Distilled water, 10 mL/kg)	Positive control (Diclofenac sodium, 50 mg/kg)	250 mg/kg	500 mg/kg	1000 mg/kg
Liver function parameters					
TP (mg/dL)	9.60±0.63	8.35±0.91	6.75± 0.62	6.42±1.35	8.98±1.29
ALB (mg/dL)	4.49±0.72	3.74±0.18	2.80±0.19	4.82±0.25	5.27±1.28
GLOB (mg/dL)	4.21±1.10	4.49±1.20	3.97±0.98	4.29±2.11	3.29±0.91
AST (U/I)	82.89±15.51	68.26±3.77	77.40±2.12	78.39±7.39	96.18±5.94
ALT (U/I)	39.30±3.13	28.90±4.21	41.80±2.29	43.28±6.48	32.45±4.38
ALP (U/I)	222.60±79.35	259.00±34.38	249.89±75.39	352.90±45.76	247.49±66.31
Renal function parameters					
CRT (mg/dL)	0.63±0.12	1.52±0.12	0.54±0.23	0.44±0.17	0.54±0.19
Urea (mg/dL)	67.27±4.23	56.20±3.32	74.10±3.41	79.24±6.24	63.36±4.29

TP, Total protein; ALB, albumin; GLOB, globulin; CRT, Creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

CONCLUSION

This study is a follow up to that of anti-inflammatory potential of *J. pinnata* leaf, and compliments most of the earlier findings. The tested agents from *J. pinnata* stem bark inhibited protein denaturation more effectively in the BSA model, and thus may have anti-inflammatory effect which was less than that of the standard drug, Diclofenac sodium®. From this present study, MeOH extract was comparable to standard drug in most of the haematological indices, some biochemical parameters and reduction of rat paw oedema, was more active in ESR inhibition and incomparable in inhibition of protein denaturation. and hence could serve as an alternative plant remedy in the treatment of rheumatoid diseases. AQ fraction was less potent than extract in inhibition of protein denaturation and may not require further phytochemical investigation. Use of medicinal plants with anti-inflammatory effects like *J. pinnata* stem bark in this study, would be preferred to the non-steroidal anti-inflammatory drugs with a history of severe side effects. This present investigation has provided an update to the compendium of anti-inflammatory plants of Nigeria.

Conflict of interest

The authors declare no conflict of interest in this work.

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Author Contributions
AAG was the research leader and wrote the manuscript, OPO and AOA undertook the experiments.

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