Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Antioxidant, Anti-inflammatory, Analgesic Properties, and Phytochemical Characterization of Stem Bark Extract and Fractions of *Anthocleista nobilis*

Kenneth Gerald Ngwoke, Amaka Godsaveus Akwagbulam, Ernest Oghenesuvwe Erhirhie[1], Daniel Lotanna Ajaghaku[2], Festus Basden Chiedu Okoye, Charles Okechukwu Esimone[3]

Departments of Pharmaceutical and Medicinal Chemistry, [1]Pharmacology and Toxicology and [3]Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, [2]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria

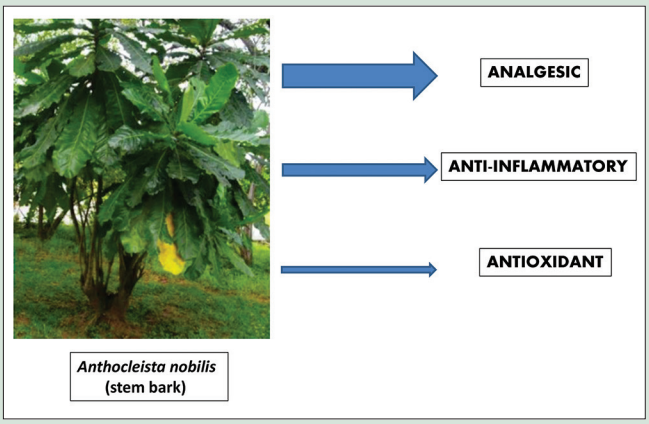
ABSTRACT

Background: *Anthocleista nobilis* (*Loganiaceae*) is used by Mbano people of Imo State, Nigeria, for the treatment of various ailments **Objective:** The aim of this study is to evaluate the antioxidant, anti-inflammatory, and analgesic properties of the methanol extract, fractions, and subfractions of *A. nobilis*. **Materials and Methods:** The powdered stem bark was extracted with methanol and sequentially fractionated into n-hexane, ethyl acetate, and butanol fractions. The constituents of the fractions were analyzed using high-pressure liquid chromatography (HPLC), and the components were identified by dereplication. Antioxidant potential of the extracts and fractions was investigated using 2,2-diphenyl-1-picrylhydrazyl free-radical scavenging method. Anti-inflammatory and analgesic activities of the extract and fractions were also investigated using xylene-induced inflammation and acetic acid-induced writhing models, respectively. **Results:** A total of five compounds isovitexin (R_t = 18.77 min), isovitexin-2″-O-xyl (R_t = 19.68 min), p-Hydroxybenzoic acid (R_t = 11.88 min), Sarasinoside L (R_t = 19.64 min), isovitexin (R_t = 18.77), and apigenin monoglycoside (R_t = 19.64 min) were identified by HPLC analysis and dereplication. The ethyl acetate fraction and subfraction elicited the best anti-inflammatory activity. The ethyl acetate subfraction also inhibited acetic acid-induced pain by 79% and 85.0% at the doses of 100 mg/kg and 200 mg/kg, respectively, which was better than 71.1% and 81.3% observed for diclofenac at similar doses. **Conclusion:** *A. nobilis* could be a potential source of anti-inflammatory and analgesic lead compounds.

Key words: Analgesic, *Anthocleista nobilis*, anti-inflammatory, Antioxidant, high-pressure liquid chromatography analysis

SUMMARY

- The extract, fractions and subfractions of Anthocleista nobilis were screened for antioxidant, anti-inflammatory and Analgesic properties in vitro and in mice models. Some of the components were identified by dereplication after HPLC analysis. The results demonstrated potent anti-inflammatory and analgesic property of the extracts and fractions. The dereplication analysis also identified vitexin and related compounds.



Abbreviation used: HPLC: High performance Liquid Chromatography; VLC: Vacuum Liquid Chromatography; DPPH: 2,2-Diphenyl-1-pycrylhydrazine; SPSS: Statistical Package for the Social Sciences; ROS: Reactive Oxygen Species

Correspondence:
Dr. Kenneth Gerald Ngwoke,
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
E-mail: kg.ngwoke@unizik.edu.ng

DOI: 10.4103/pr.pr_73_17

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Oxidative stress plays a significant role in the pathogenesis of several human diseases including inflammatory conditions, neurological disorders, cardiovascular complications, and cancers among other ailments.[1]

Management of pains and inflammatory-mediated diseases with conventional therapeutic agents has not always produced the most desired result because of the numerous adverse effects such as gastric erosion and liver toxicity associated with some of the agents.[1]

Following the limitations posed by existing and mostly synthetic agents, the search for alternative therapies from natural sources including medicinal plants has received greater attention recently.[2] Historically, medicinal plants has played significant role in the management of numerous disease conditions, especially in the rural areas, due to their availability and affordability.[3] Interestingly, good numbers of modern drugs were derived from natural sources, mostly based on their ethnomedicinal relevance.[3]

Anthocleista nobilis (G. Don) belonging to the family *Loganiaceae*, is a small to medium-sized tree growing up to 30-m tall. It is commonly

Cite this article as: Ngwoke KG, Akwagbulam AG, Erhirhie EO, Ajaghaku DL, Okoye FB, Esimone CO. Antioxidant, Anti-inflammatory, Analgesic Properties, and Phytochemical Characterization of Stem Bark Extract and Fractions of *Anthocleista nobilis*. Phcog Res 2018;10:81-7.

found in tropical African habitats such as the Mascarene Islands and Madagascar as well as Southern, Western, and Eastern part of Nigeria. The bark is smooth and pale grey. The inner bark is cream-yellow and granular, whereas the twig has 2 spines above the leaf axis. The leaves are opposite, crowded at the end of branches, and petiole is 1–6 cm long.[4]

It is commonly called candelarum, cabbage tree, cabbage palm, or palma christi in English language. It is also locally known as Uko nkirisi in Igbo language. Conventionally, *A. nobilis* is used in the treatment of fever, stomach ache, diarrhea, and gonorrhea. It is also used as strong purgative, diuretic, and as poultice for treating sores in parts of West Africa.[5] It is used as vapor bath for the treatment of leprosy, venereal diseases, and dysmenorrheal. Its root decoction is usually taken to regulate menstruation and also as an abortifacient. In Mbano community in Imo State, Nigeria, the root bark decoctions are mostly used in the treatment of diabetes mellitus, gastrointestinal worms, malaria, and jaundice.[5]

The studies had shown that the root bark of *A. nobilis* possessed anti-diabetic activity,[5] antiviral and anti-plasmodial activities,[6,7] anti-Leishmanial activity,[8] antibacterial, antioxidant activity, and wound healing properties.[9] Recent studies has shown that its acetone and methanol stem bark extracts possess moderate free-radical scavenging activities.[10] Phytochemical analyses also revealed the presence of polyphenols, flavonoids, tannins, and triterpenoids, coumarins and saponosides.[10,11] The present study was designed to evaluate the anti-inflammatory and analgesic properties of methanol extract, fractions, and ethyl acetate chromatographic fractions of *A. nobilis* which to the best of our knowledge has not been previously reported. We also report for the first time the identification of Vitexin and it is derivatives in the stem bark of *A. nobilis*.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade methanol, n-hexane, ethyl acetate, and n-Butanol were obtained from JHD, Shantou, Guangdong, China), 2,2-diphenyl-1-picrylhydrazyl (DPPH), xylene, and ascorbic acid were obtained from Sigma-Aldrich, Germany. Indomethacin and Diclofenac sodium were obtained from Hovid (Bhd) Pharmaceuticals, Malaysia. All laboratory reagents were freshly prepared when required.

Plant collection and authentication

Stem barks of *A. nobilis* were collected from Ezza Community in Ebonyi State Nigeria, in March, 2014. They were identified and authenticated by Mrs. Emezie A, a plant taxonomist in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria. A voucher specimen PHC0098 was deposited in the herbarium of the same Department for reference purposes.

Experimental animals

Swiss Albino mice (25–30 g) were obtained from the animal house of the Department of Pharmacology, Nnamdi Azikiwe University, Agulu. The animals were housed in standard laboratory conditions of 12 h light, room temperature, and 40%–60% relative humidity. They were allowed free access to food (Guinea feeds Nigeria Ltd) and water *ad libitum*. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (National Institute of Health (2011) pub No: 85-23).

Preparation of plant extract

Air-dried stem bark of *A. nobilis* ground coarse consistency using a locally fabricated mechanical grinder. Exactly 900 g of the pulverized stem bark was macerated in methanol with intermittent shaking for 72 h. The extract was removed every 12 h and fresh solvent introduced. At the end of 72 h, the extracts were pooled together, strained through muslin cloth, and filtered with filter paper (Whatman No. 1). The filtrate was evaporated to dryness using rotary evaporator (RE300 Model, United Kingdom) at 40°C. The extract was stored at 4°C until when required.

Liquid-liquid partitioning

The methanol extract of *A. nobilis* was sequentially partitioned n-hexane, ethyl acetate, and butanol. All the fractions obtained were filtered using Whatman No. 1 filter paper and were concentrated with a rotary evaporator at 40°C. The fractions obtained were stored at 4°C. The fractions were all subjected to bioactivity testing and high-performance liquid chromatography (HPLC) analysis. Based on the result of the bioactivity testing of the fractions, ethyl acetate fraction (1.39 g) was further fractionation using vacuum liquid chromatography (VLC).

Vacuum liquid chromatographic separation of ethyl acetate fraction

The ethyl acetate fraction (1.39 g) mixed with 20 g of silica gel (200–400, mesh) was loaded on top of the glass column already packed with silica gel (mesh size; 200–400). The sample was gradually eluted with mobile phase in increasing order of polarity from nonpolar to polar solvents. Different fractions were collected and concentrated using rotary evaporator.

Analytical high-performance liquid chromatography analysis

Each of the dried crude extract and fractions (2 mg) was dissolved 2 mL of HPLC grade methanol and the mixture was centrifuged at 3000 rpm for 5 min. Then, 100 µL of the dissolved samples was transferred into HPLC vials containing 500 µL of HPLC grade methanol.

HPLC analysis was carried out on the samples with a Dionex P580 HPLC system coupled to a photodiode array detector. Detection was at 235, 254, 280, and 340 nm. The separation column (125 mm × 4 mm; length × internal diameter) was prefilled with Eurosphere C-18 (Knauer, Germany), and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as the eluent. Compounds were detected using diode array and identified based on similarity with data in the inbuilt library.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The DPPH free-radical scavenging activities of the extract, fractions, and ethyl acetate chromatographic fractions of *A. nobilis* was evaluated by the method described by Patel and Patel[12] with some modifications. Half milliliter (0.5 mL) of DPPH solution (0.6 mM) was added to 0.5 mL of different concentrations of the extract and fractions (15.63, 31.25, 62.25, 125, 250, 500, and 1000 µg/mL). The volume of the solution was adjusted with methanol to a final volume of 5 mL. The mixture was incubated in the dark for 30 min at room temperature and absorbance of the mixtures was obtained at 517 nm using VIS spectrophotometer (Model 752, China). All the tests were performed in duplicate and ascorbic acid was used as standard. The absorbance of the control (containing 0.5 mL of DPPH solution and 4.5 mL of methanol) was used to calculate the free radical scavenging activities. The percentage radical scavenging potentials of the extracts, fractions and standard (ascorbic acid) were calculated using the equation below.

$$\text{Percentage of inhibition of free radical} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

Where A_0 is the absorbance of the control, A_t is the absorbance of the test/standard.

The 50% inhibitory concentration (IC_{50}) was determined from a plot of percentage scavenging potentials against concentration.

Anti-inflammatory activity of extracts and fractions

The effect of the extract on xylene-induced acute topical inflammation was evaluated with modification of the method adopted by Atta and Alkohafi.[13] Adult Swiss albino mice (5 per group) were used for the study. The extract and fraction treatment groups received 250 and 500 µg/anterior surface of the right ear. Negative control group received the vehicle, 50 µL of 5% Tween-80, whereas the positive control group received 250 and 500 µg of Indomethacin/anterior surface of the right ear. Immediately after treatment, topical inflammation was induced by application of 50 µL of xylene on the posterior surface of the same ear. Two hours after induction of inflammation, the mice were sacrificed by cervical dislocation and sections of both (right and left) ears were punched out with the aid of circular cork borer (4 mm diameter) and were weighed with the aid of analytical weighing balance (Ohaus, China). Edema was quantified as the weight difference between the two earplugs. The anti-inflammatory activity was evaluated as percentage edema reduction/inhibition in the treated animals relative to control animals[14] using the relation:

Percentage edema reduction (%) =1− (RET− LET/REC − LEC) × 100

where,

RET = Mean weight of right earplug of treated animals; LET = Mean weight of left earplug of treated animals; REC = Mean weight of right earplug of control animals; LEC = Mean weight of left earplug of control animals.

Anti-inflammatory activity of ethyl acetate chromatographic fractions

Similar method and procedure above were used in the screening of the anti-inflammatory activity of various ethyl acetate chromatographic fractions from ethyl acetate main fractions.

Analgesic activity on extract, ethyl acetate fraction

This study was carried out using acetic acid-induced abdominal writhing reflex pain model as described by Smahane *et al.*[15] with some modifications. Adult mice (25–30 g) were fasted 12 h before the experiment and were randomly divided into 4 groups of 4 mice each, and treated as follows, group 1 (negative control group) received 10 mL/kg normal saline, group 2 and 3 (positive control groups) received 100 and 200 mg/kg of diclofenac sodium; groups 4 and 5 received 100 and 200 mg/kg of *A. nobilis* methanol extract, whereas group 6 and 7 received 100 and 200 mg/kg of ethyl acetate fraction of *A. nobilis* using gastric gavages. One hour after administration, 10 mL/kg of 0.6% glacial acetic acid was administered intraperitoneally (I. P) to all the mice to induce pain. The pain responses by animals characterized by abdominal writhing was counted were recorded and analgesic activity was calculated using the following equation;

$$\text{Analgesic activity} = \frac{\text{Abdominal writhing in control - Abdominal writing in test group}}{\text{Abdominal writing in control}} \times \frac{100}{1}$$

Statistical analysis

Results were presented as mean ± standard error of mean. Statistical comparisons among and between group means was performed using one-way analysis of variance followed by *post hoc* Turkey's test for multiple analyses using Statistical Package for the Social Sciences (SPSS-20, IBM corporation, USA). *P* values ($P < 0.05$) were considered to be statistically significant. IC_{50} of the extracts and fractions were calculated from the graph of regression equation using Microsoft Excel, 2010.

RESULTS

High-performance liquid chromatography identification of compounds from extract and fractions of *Anthocleista nobilis*

The HPLC chromatogram of the methanol extract [Figure 1] revealed the presence of isovitexin (R_t = 18.77 min) and isovitexin-2"-O-xyl (R_t = 19.68 min. N-hexane fraction [Figure 2] revealed the presence of p-Hydroxybenzoic acid (R_t = 11.88 min) and Sarasinside L (R_t = 19.64 min). Ethyl acetate fraction [Figure 3] revealed isovitexin (R_t = 18.77), whereas butanol fraction [Figure 4] revealed the presence of apigenin monoglycoside (R_t = 19.64 min).

Antioxidant result of crude extract and various fractions

All the samples tested showed radical scavenging activity against DPPH. Methanol extract (AC1), n-hexane fraction (AC2), ethyl acetate fraction (AC3), butanol fraction (AC4) manifested antioxidant activities with IC_{50} values of 528, 570.5, 220, and 529.4 µg/mL, respectively. Ethyl acetate fraction showed moderate antioxidant activity compared to the other fraction. However, when compared with the standard ascorbic acid (IC_{50} 23.39 µg/mL), the antioxidant activity of ethyl acetate fraction was about ten-fold less. The order of scavenging activity of the test samples was AC3 > AC1 > AC4 > AC2 [Table 1].

Antioxidant result of ethyl acetate chromatographic fraction

From Table 2, AV2 showed the highest antioxidant activity ($IC_{50.}$ 220 µg/mL) after ascorbic acid ($IC_{50.}$ 60 µg/mL) while AV1 showed the least activity with ($IC_{50.}$ 1564.1 µg/mL). The order of

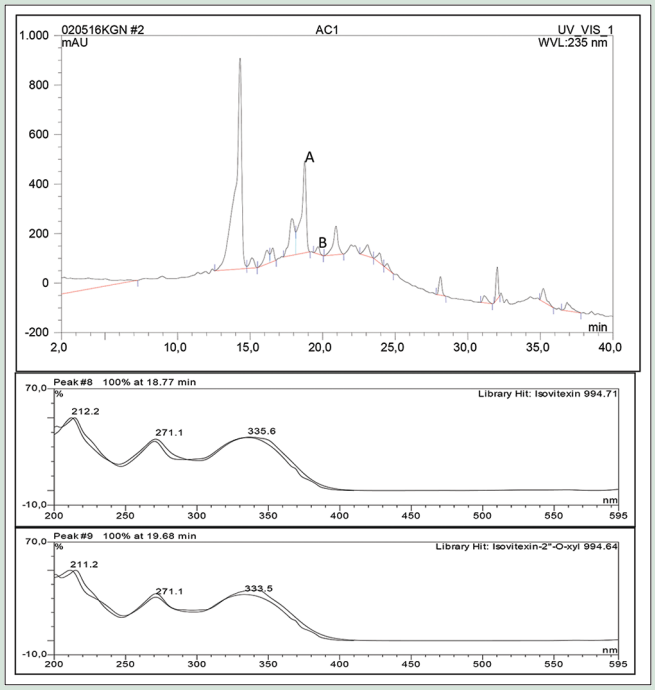


Figure 1: High-pressure liquid chromatography chromatogram of methanol extract and ultraviolet chromatogram showing the presence of isovitexin fraction and ultraviolet chromatogram of triterpene acetate. E = Peak 4

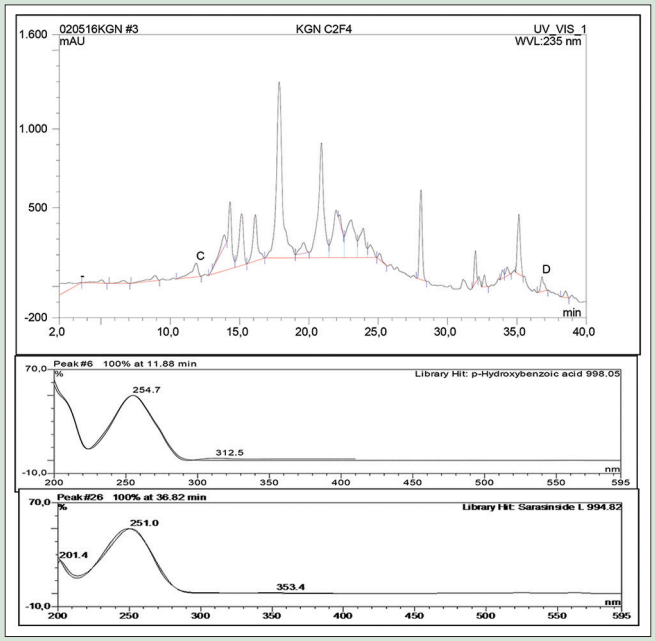


Figure 2: High-pressure liquid chromatography chromatogram of n-hexane fraction (AC2) and UV chromatogram of p-Hydroxybenzoic acid and UV chromatogram of Sarasinside L. C: p-Hydroxybenzoic acid; D: Sarasinside L

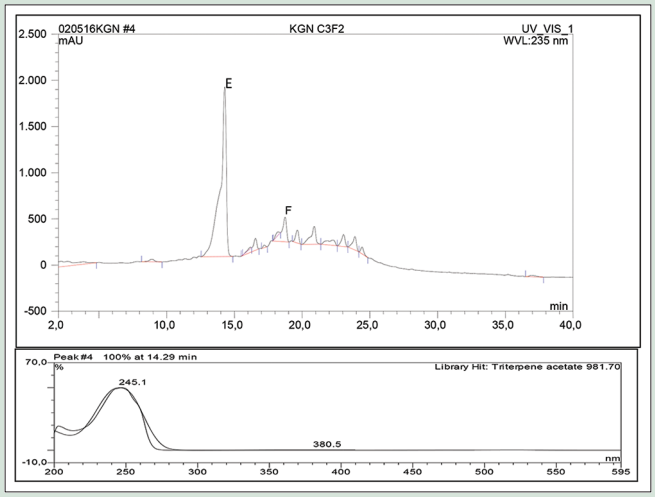


Figure 3: High-pressure liquid chromatography chromatogram of ethyl acetate fraction (AC3) and ultraviolet chromatogram of triterpene acetate. E: Peak 4

the scavenging activity of the ethyl acetate subfraction include AV2 > AV7 > AV9 > AV5 > AV10 > AV8 > AV1 [Table 2].

Effect of methanol extract and fractions on xylene-induced topical inflammation

The results presented in Table 3 demonstrate that the methanol extract and fractions exhibited significant ($P < 0.05$) anti-inflammatory activity in a nondose-dependent manner. Ethyl acetate fraction showed the highest activity, and at doses of 250 and 500 µg/ear decreased the ear edema rate at 83.49 and 68.40%, respectively, similar to indomethacin. The ethyl acetate, n-hexane, and butanol fractions had better activity at a lower dose of 250 µg/ear compared to 500 µg/ear.

Anti-inflammatory activity of ethyl acetate chromatographic fractions of *Anthocleista nobilis*

The results presented in Table 4 showed that ethyl acetate chromatographic fraction elicited a significant ($P < 0.05$) anti-inflammatory activity. Subfraction AV9 showed the highest activity by decreasing ear edema by 55.03% and 48.52% at doses of 250 µg/ear and 500 µg/ear respectively [Table 4].

Effects of methanol extract and ethyl acetate fraction of *Anthocleista nobilis* on acetic acid-induced writhing reflex of on mice

The oral administration of 100 mg/kg and 200 mg/kg of methanol extract significantly ($P < 0.05$) inhibited writhing response induced by acetic acid, giving a percentage inhibition of 82.4% and 75.7%, respectively, as compared with the negative control [Table 5]. Significant ($P < 0.05$) inhibition was also recorded with the ethyl acetate fraction. At the same doses, the activities of the extract and ethyl acetate fractions were comparable to diclofenac sodium.

Table 1: Result of 2,2-diphenyl-1-picrylhydrazyl scavenging activity of extract and various fractions of *Anthocleista nobilis*

Extracts/fractions	Inhibition concentration, IC_{50} (µg/mL)
Crude extract	528
n-hexane	570.5
Ethyl acetate	220
Butanol	529.4
Aqueous	365.2
Ascorbic acid	23.4

Table 2: Result of 2,2-diphenyl-1-picrylhydrazyl scavenging activity of ethyl acetate chromatographic fractions of *Anthocleista nobilis*

VLC subfractions	Percentage inhibition/concentration IC_{50} (µg/mL)
AV1	1564.1
AV2	220
AV5	574.71
AV7	351.92
AV8	608.21
AV9	571.61
AV10	598.62
Ascorbic acid	60.00

VLC: Vacuum liquid chromatography

Effects of ethyl acetate chromatographic fractions of *Anthocleista nobilis* on acetic acid-induced writhing reflex on mice

Table 6 shows the effect of the ethyl acetate chromatographic fraction on acetic acid-induced writhing in mice. At 100 and 200 mg/kg doses, the descending order of inhibition by the various fractions showed that AV9 (79 and 85%) > AV10 (67.4 and 75.9%) >AV8 (53.5 and 54.5%) >, AV5 (26.2 and 61.0%) >AV7 (16.0 and 37.4%) >AV1 (69.5 and 20.3%). Diclofenac sodium caused inhibition of 71.1% and 81.3%, lesser than that of AV9.

DISCUSSION

Oxidative stress, inflammation, and pain are closely associated processes that can be simultaneously present in many pathological conditions.[16]

Table 3: Effect of methanol extract and fractions of *Anthocleista nobilis* on xylene-induced topical inflammation

Treatment	Dose (µg/ear)	Mean left ear (mg)	Mean right ear (mg)	Mean edema (mg)	Inhibition (%)
Methanol extract	250	0.40±0.02	0.77±0.09	0.37±0.09	11.32
	500	0.53±0.03	0.80±0.14	0.27±0.11	15.09
n-hexane fraction	250	0.39±0.02	0.68±0.11	0.29±0.09	32.55
	500	0.43±0.02	0.74±0.08	0.31±0.08	30.19
Ethyl acetate fraction	250	0.40±0.02	0.50±0.04	0.10±0.04*	83.49
	500	0.41±0.02	0.54±0.05	0.13±0.05*	68.4
Butanol fraction	250	0.45±0.01	0.68±0.07	0.23±0.07	44.34
	500	0.45±0.01	0.69±0.12	0.24±0.12	40.09
Aqueous fraction	250	0.41±0.01	0.65±0.09	0.24±0.08	43.34
	500	0.42±0.01	0.65±0.07	0.23±0.08	45.28
Indomethacin	250	0.46±0.02	0.52±0.03	0.06±0.02*	85.85
	500	0.41±0.01	0.46±0.01	0.05±0.01*	89.15
5% tween-80	50 µL/ear	0.43±0.01	0.86±0.03	0.43±0.03	

n=5; *P<0.05 compared with 5% tween-80 (vehicle)-treated group

Table 4: Effect of ethyl acetate chromatographic fraction of *Anthocleista nobilis* on xylene-induced topical inflammation

Treatment	Dose (µg/ear)	Mean left ear (mg)	Mean right ear (mg)	Mean edema (mg)	Inhibition (%)
AV1	250	0.58±0.02	0.91±0.06	0.33±0.06	22.49
	500	0.66±0.04	0.96±0.02	0.31±0.04	27.81
AV5	250	0.53±0.04	0.69±0.07	0.16±0.07*	63.91
	500	0.53±0.05	0.81±0.14	0.28±0.10	33.14
AV7	250	0.69±0.08	1.00±0.07	0.31±0.08	26.63
	500	0.72±0.12	1.06±0.11	0.34±0.03	21.31
AV8	250	0.52±0.04	0.76±0.08	0.24±0.06*	44.97
	500	0.60±0.05	0.88±0.05	0.28±0.09	33.14
AV9	250	0.63±0.03	0.81±0.09	0.18±0.07*	55.03
	500	0.54±0.04	0.75±0.08	0.21±0.07*	48.52
AV10	250	0.48±0.04	0.80±0.06	0.32±0.05	25.55
	500	0.40±0.00	0.94±0.02	0.54±0.02	15.38
Indomethacin	250	0.47±0.02	0.53±0.04	0.05±0.02*	87.62
	500	0.42±0.01	0.46±0.01	0.04±0.01*	89.94
5% tween 80	50 µg/ear	0.44±0.02	0.86±0.03	0.42±0.04	

AV1 (*n*=5); *P<0.05 compared with 5% tween 80 (vehicle) treatment

Table 5: Effects of methanol extract and ethyl acetate fraction of *Anthocleista nobilis* on acetic acid-induced writhing reflex of on mice

Treatment	Dose (mg/kg)	Mean writhing reflex±SEM	Inhibition (%)
Methanol extract	100	10.50±4.09*	82.4
	200	8.25±2.36*	77.5
Ethyl acetate fraction	100	10.75±3.12*	73.8
	200	12.25±4.39*	77
Diclofenac sodium	100	13.50±4.13*	81.3
	200	8.75±3.47*	71.1
5% tween 80	5 mL/kg	46.75±10.44*	

n=5; *P<0.05 compared with 5% Tween-80 (vehicle-treated control) group. SEM: Standard error of mean

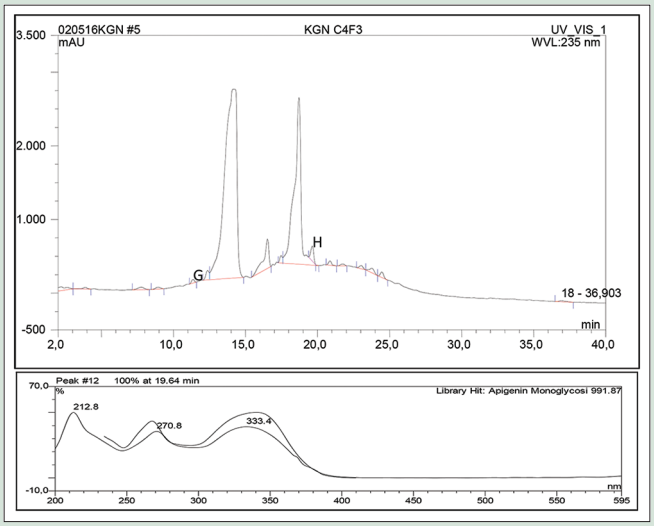


Figure 4: High-pressure liquid chromatography chromatogram of butanol fraction and ultraviolet chromatogram of apigenin monoglycoside. H: Apigenin monoglycosi (R_t = 19.64)

Substances effective against these conditions may mediate their effect through pathophysiological processes common among them. Findings from this experiment revealed that *A. nobilis* showed activity against free radicals, inflammation, and pain.

The HPLC chromatogram of the extract and fractions of *A. nobilis* stem bark showed the abundance of Vitexin (a glycoside of the flavones class) and its derivatives. Apigenin-6-C-β-D-glucoside (isovitexin) has previously been isolated from *Polygonatum odoratum* (Mill)[17] and has also been reported by Qiulan *et al.*[18] as an efficient antioxidant. A research carried out by Liu and Jan[19] revealed that isovitexin protected DNA from the Fenton reaction-induced breakage in a dose-dependent manner. Isovitexin has also been documented to have protected HL-60 cells from the ROS damage induced by the xanthine/xanthine oxidase reaction.[20] Its radioprotective effect and antihyperglycemic activity have been reported.[21,22] Isovitexin and isovitexin 2"-O-xyl compounds identified in this study may have contributed to the antioxidant and anti-inflammatory activities of *A. nobilis*.

Table 6: Effect of ethyl acetate chromatographic fraction on acetic acid-induced writhing reflex

Treatment	Dose (mg/kg)	Mean writhing reflex±SEM	Inhibition (%)
AV1	100	14.25±7.30*	69.5
	200	37.25±11.25	20.3
AV5	100	34.50±10.59	26.2
	200	18.25±5.98*	61
AV7	100	39.25±7.78	16
	200	29.25±3.86*	37.4
AV8	100	21.75±3.86*	53.5
	200	21.25±3.97*	54.5
AV9	100	11.75±2.25*	79
	200	7.00±1.08*	85
AV10	100	15.25±6.09*	67.4
	200	11.25±3.20*	75.9
Diclofenac sodium	100	13.50±4.13*	71.1
	200	8.75±3.47*	81.3
5% Tween 80	5 mL/kg	46.75±10.44*	

n=5; *P<0.05 compared with 5% Tween 80 (vehicle-treated control) group. SEM: Standard error of mean

P-hydroxybenzoic acid identified from the n-hexane fraction is a phenolic derivative of benzoic acid. Several biological activities of this compound have been reported. Merkl *et al.*[23] reported that phenolic acid such as p-Hydroxybenzoic acid possesses good antioxidant activity. p-Hydroxybenzoic acid has also been known for its anti-inflammatory activity as reported by Luecha *et al.*[24] This compound may have also contributed to the analgesic, anti-inflammatory, and antioxidant activities exhibited by *A. nobilis*.

The HPLC chromatogram of the ethyl acetate fraction revealed the presence of unidentified compounds (Peak 4 Rt. 14.29 min). From its intensity and peak area, it is clear that peak 4 is the major compound in the extract. The ethyl acetate fraction and its subfractions showed appreciable activity in all the tests carried out. Comparing the intensities of peak 4 in n-hexane and ethyl acetate fractions, the intensity of this peak is greater in ethyl acetate fraction (2000 mAU) than n-hexane fraction (1400 mAU). This suggests that peak 4 could have contributed to why ethyl acetate fraction gave the best activities among the fractions. Dereplication analysis identified peak 4 as triterpene acetate with hit of 981 which is below 990, the acceptable minimum. Further purification and spectroscopic experiment would be required to elucidate the structure and identity of peak 4.

DPPH, a stable free radical, can accept an electron or hydrogen to become a stable molecule, and it can also be used as a substrate to evaluate the antioxidant activity of compounds.[25,26] The moderate antioxidant activity of ethyl acetate could be attributed to the presence of flavonoid especially isovitexin. Polyphenolic compounds, such as flavonoid and phenolic acids, which are mostly found in plants have been reported for their biological activities, including antioxidant activity.[25] Earlier report by Ngwoke *et al.*[10] also indicated that *A. nobilis* is rich in flavonoids, terpenoids, and tannins. Interestingly, the components of the crude extract and fractions of *A. nobilis* as revealed by HPLC chromatogram had been reported for their antioxidant activities as earlier stated.

Xylene, a phlogistic agent is a useful tool in the assessment of acute inflammation. Topical application of xylene causes irritation of the living tissues, thereby leading to fluid accumulation and edema.[13] Suppression of xylene-induced topical edema by the extract and fractions is an indication that they possess anti-inflammatory activity. This activity was, however, not exhibited in a dose-dependent manner. Better activity shown at lower dose could be attributed to reduced solubility or poor permeability at higher concentration. Previous studies have also revealed that flavonoid, phenolic compounds, isovitexin, isovitexin 2"-O-xyl, apigenin glycoside, and p-Hydroxybenzoic acid possess anti-inflammatory activity due to their inhibitory effects against mediators of inflammation.[24,27] The presence of these compounds could account for the anti-inflammatory activity of *A. nobilis*.

Acetic acid-induced pain is an experimental model of evaluating peripherally acting analgesics. The pain response is thought to be mediated by peritoneal mast cells and the prostaglandin pathways.[28] The organic acid has also been known to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to analgesics.[29] Compounds such as flavonoids and steroids have been shown to possess anti-inflammatory and analgesic activity as reported by Pritam *et al.*[30] The potent analgesic activity of *A. nobilis* may be derived from its anti-inflammatory properties since the reduction in acute inflammation also mediates reduction in pain.[31]

CONCLUSION

This study had revealed that crude extract, ethyl acetate fraction, and subfraction from the stem bark of *A. nobilis* showed potent anti-inflammatory and analgesic activities that could be attributed to the active compounds as revealed by the HPLC analysis. Outstandingly, the dose-dependent and potent inhibition of the acetic acid-induced writhing which was better than the inhibition observed for the positive control suggests that the extract has great potential for use as analgesic and anti-inflammatory agent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Genesh G, Saurabh M, Sarada NC. Antioxidant and anti-inflammatory activities of the methanolic leaf extract of traditionally used medicinal plants *Mimusops elengi L*. Pharm Sci Res 2013;5:125-30.
2. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn. – An *in vitro* approach. JKSUS 2014;26:267-75.
3. Das B, Ferdous T, Mahmood QA, Hannan JM, Bhattacharjee R, Das BK. Antiinociceptive and anti-inflammatory activity of the bark extract of *Plumeria rubra* on laboratory animals. Eur J Med Plants 2013;3:114-26.
4. Dokosi OB. Herbs of Ghana. Accra: Ghana University Press; 1998. p. 615-23.
5. Madubunyi II, Asuzu IU. Pharmacological screening of *Anthocleista nobils* root bark. Int J Pharmacogn 1995;34:28-33.
6. Ayodele PO, Okonko IO, Odu NN, Banso A. Antiviral effect of *Anthocleista nobilis* root extract on the biochemical indices of poultry fowls infected with New castle Disease Virus (NDV). Ann Biol Res 2012;3:20-30.
7. Ogbuehi IH, Ebong OO, Asuquo EO, Nwauche CA. Evaluation of the antiplasmodial activity of the methanolic root extracts *Anthocleista nobils* G. Don, *Nauclea latifolia* smith and *Napoleona imperialis* p. Beauv. Br J Pharmacol 2014;5:75-82.
8. Salami OA, Fafunso MA, Choudhary MI, Atta UR. Toxicological screening of the ethanol root bark extracts of two Anthocleista species World Appl Sci J 2015;33:1164-70.
9. Annan K, Dickson R. Evaluation of wound healing action of Hoslundia opposite Vahl, *Anthocleista nobils* G. Don. and *Balanites aegyptica* L. J Sci Technol 2008;28:26-35.
10. Ngwoke KG, Anusi IA, Eze PM, Okezie UM, Abba CC, Abonyi DO. Phytochemical and antioxidant properties of stem bark extracts of *Anthocleista nobilis*. Eur J Med Plants 2015;8:107-11.
11. Sima O, Obame EL, Ondo JP, Zongo C, Nsi SE, Traore SA. Ethnotherapy study, phytochemical screening and antioxidant activity of *Antrocary klaineanum*, pierre and *Anthocleista nobils* G.Don. Medicinal plants from Gabon. IJAR 2015;3:812-9.

12. Patel A, Patel A, Patel A, Patel NM. Determination of polyphenols and free radicals scavenging activity of *Tephrosia purpurea* Linn. Leaves (Leguminosae). Pharmacogn Res 2010;2:152-8.
13. Atta AH, Alkohafi A. Antinociceptive and anti-inflammatory effects of some Jordanian medicinal plants extracts. J Ethnopharmacol 1998;60:117-24.
14. Asuzu IU, Sosa S, Della L. The anti-inflammatory activity of *Icacina trichanthatuber*. Phytomedicine 1999;6:267-72.
15. Smahane B, Latifa EM, Mouna B, Ahmed O, Sanae A, Maroua K, *et al*. Phytochemical screening, anti-inflammatory and analgesic activities of formulation cream of silene vulgaris. Res J Med Plant 2016;10:150-8.
16. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid Med Cell Longev 2016;2016:5698931.
17. Chunsriimyatav G, Margit G, Dumaa M, Regdel D, Arndt WS, Hans-Joachm K. Flavonoidglycosides from the aerial parts of *Polygonatum odoratum* (Mill) growing in Mongolia. Open Nat J 2015;8:1-7.
18. Qiulan H, Yiran G, Rao F, Tong P, Yuting Z, Fang C. Antioxidant activity of flavonoids from leaves of *Jatropha curcas*. ScienceAsia 2014;40:193-7.
19. Liu F, Jan K. DNA damage in arsenitic-and cadmium-treated bovine aortic endothelial cells. Free Radic Biol Med 2000;28:55-63.
20. Chang WS, Chang YH, Lu FJ, Chang HC. Inhibitory effects of phenolics on xanthine oxidase. Anticancer Res 1994;14:501-6.
21. Fu Y, Zu Y, Liu W, Hou C, Chen L, Li S, *et al*. Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. J Chromatogr A 2007;1139:206-13.
22. Folador P, Cazarolli H, Gazola AC, Reginatto FH, Schenkel EP, Silva FR. Potential insulin secretagogue effects of isovitexin and swertisin isolated from *Wilbrandia ebracteata* roots in non-diabetic rats. Fitoterapia 2010;81:1180-7.
23. Merkl R, Hradkova I, Filip V, Smidrkal J. Antimicrobial and antioxidant properties of phenolic acids alkyl esters. Czech J Food Sci 2010;28:275-9.
24. Luecha P, Umehara K, Miyase T, Noguchi H. Antiestrogenic constituents of the Thai medicinal plants *Capparis flavicans* and *Vitex glabrata*. J Nat Prod 2009;72:1954-9.
25. Lompo M, Dubois J, Guissou IP. *In vitro* preliminary study of free radical scavenging activity of extract from *Khaya senegalensis* A. Juss. (*Meliaceae*). J Biol Sci 2007;7:677-80.
26. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. J Nat Prod 2001;64:892-5.
27. Vo TN, Phi LT, Lam TP, Lawrence MV, Phung NN, Kim PP. Lignans and Triterpenes from the root of *Pseuderanthemum carruthersii var atropurpureum*. Chem Pharm Bull 2012;60:1125-33.
28. Voilley N. Acid-sensing ion channels (ASICs): New targets for the analgesic effects of non-steroid anti-inflammatory drugs (NSAIDs). Curr Drug Targets Inflamm Allergy 2004;3:71-9.
29. Adzu B, Amos S, Kapu SD, Gamaniel KS. Anti-inflammatory and anti-nociceptive effects of *Sphaeranthus senegalensis*. J Ethnopharmacol 2003;84:169-73.
30. Pritam SJ, Amol T, Sanjay BB, Sanjay JS. Analgesic activity of *Abelmoschus monihot* extracts. Int J Pharmacol 2011;7:716-20.
31. Sawadogo WR, Boly R, Lompo M, Some N, Lamien CE, Guissou IP, *et al*. Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. Int J Pharm 2006;2:435-8.