Int. J. Plant Bas. Pharm. (2023) 3(1), 32-40

INTERNATIONAL JOURNAL OF PLANT BASED PHARMACEUTICALS

https://ijpbp.com

RESEARCH ARTICLE

OPEN ACCESS

Ruellia simplex C. Wright (Acanthaceae): Antinociceptive, anti-inflammatory, and antidiabetic activities of a novel fatty acid isolated from its leaf extract

Cletus Anes Ukwubile[a*], Henry Nettey[b], Troy Salvia Malgwi[a], Nnamdi David Menkiti[c]

[a] *University of Maiduguri, Faculty of Pharmacy, Department of Pharmacognosy, Nigeria*
[b] *University of Ghana, School of Pharmacy, Department of Pharmaceutics and Pharmaceutical Microbiology, Legon, Accra, Ghana*
[c] *Ahmadu Bello University, Faculty of Physical Sciences, Department of Chemistry, Zaria, Nigeria*

ARTICLE INFO

Article History:

Received: 12 August 2022
Revised: 27 September 2022
Accepted: 05 October 2022
Available online: 07 October 2022

Edited by: B. Tepe

Keywords:

Ruellia simplex
Antinociceptive
Anti-inflammatory
Antidiabetics
Fatty acid

ABSTRACT

Ruellia simplex is a medicinal plant whose leaf is used to treat pains, inflammation, and diabetes in Nigeria. The current study was undertaken to determine the antinociceptive (analgesics), anti-inflammatory, and antidiabetic activities of a novel fatty acid isolated from the leaf extract of *R. simplex*. Isolation of a novel fatty acid from the most active fraction was carried out on silica gel column chromatography while, antinociceptive, anti-inflammatory, and antidiabetic activities of the isolated compound were evaluated by acetic acid, carrageenan, and alloxan-induced animal models respectively. The chemical structure of the new compound was elucidated by FT-IR, NMR, GC-MS, and LC-MS. The isolated fatty acid showed inhibition of pains by decreasing abdominal writhing in mice in dose dependent fashion as well as reduced paw volume in the carrageenan-induced paw edema in rats at IC_{50} = 12.5 ± 1.08 µg/ml and 10.21 ± 1.02 µg/ml, respectively, whereas the antidiabetic activity showed a dose dependent reduction in blood sugar levels with IC_{50} = 6.02 ± 0.01 µg/ml. The compound showed the following features: R-COOH functional group at 3327 wavelength cm^{-1} by FTIR; EI-MS $[M]^{+*}$ at *m/z* 467, peak area 62.231% and RT 14.086 min by GC-MS; singly charged fragments at *m/z* 116.1 and *m/z* 465.1, RT 1.31 min by LC-MS and eight proton signals consisting of singlets and multiplets (^{1}H), thirty carbon atoms (^{13}C) NMR data. From the study, the novel fatty acid from *R. simplex* extract was potentially active for the treatment of pains, inflammation, and diabetes.

1. Introduction

The discovery of drugs from indigenous herbal plants has progressed with many civilizations. This is because medicinal plants have been used to treat different types of diseases for decades (Tabuti et al., 2012). In most cases, the science or mechanism for the use of these plants is not clear or known to herbalists or traditional medicine practitioners, yet the scientific validation for the use of most of these plants has been done only in recent times (Boadu & Asase, 2017). Moreover, the significant advances in experimental and molecular biology techniques applied to these plants have paved the way for pharmacognosist and natural products researchers to find out the potential use of secondary metabolites to treat or manage an array of diseases such as cancers, hypertension, diabetes, ulcers, pains, inflammation, infections, among other diseases (Schippmann et al., 2006).

For instance, pain or analgesia is characterized as an unwanted experience and nervous agitati-

Reviewed by:
Yavuz Selim Cakmak: Aksaray University, Aksaray, Türkiye
Menderes Suicmez: Hitit University, Corum, Türkiye

* Corresponding author(s):
E-mail address:
doccletus@yahoo.com
caukwubile@unimaid.edu.ng
(C. A. Ukwubile)
e-ISSN: 2791-7509
doi: https://doi.org/10.29228/ijpbp.13

on with either short or prolonged tissue degeneration. All analgesic drugs selectively exert pain-relieving effects without pronounced change of consciousness by acting on the central nervous system or the peripheral pain receptors (Belachew et al., 2020; Nguyen et al., 2020). Despite the use of these analgesics as pain reduction agents, some of them have been classified recently as proton pump inhibitors (PPIs) indicating their beneficial off-target effects. Similarly, inflammation is an immunological response by the body against infection. For example, the febrile response is a classical sign of an inflammatory response to an invading harmful stimulus involving several mediators such as interleukins (Yeshwante et al., 2009). In addition, inflammation is usually followed by pain. For this reason, nonsteroidal anti-inflammatory drugs (NSAIDs) are the standard treatment for pains and inflammation because they inhibit the activity of cyclooxygenase enzymes, which play crucial roles in the production of prostaglandins (PGAs); endogenous mediators known to initiate pains and inflammation. The use of these NSAIDs has increased the risk of anomalies in some organs and/or tissues in the body such as the liver, kidney, GIT, and heart (Padmanabhan & Jangle, 2012; Ukwubile et al., 2021). Hence, natural product researchers have been looking for alternative herbal treatments with low side effects.

On the other hand, diabetes is a disease characterized by high blood glucose levels (known as hyperglycemia). The disease is usually associated with frequent urination and is occasionally accompanied by high blood pressure resulting in about 40% of deaths annually globally (Ibrahim et al., 2014; Ojo et al., 2018). Currently, the exact number of people living with type 2 diabetes in Nigeria has doubled in the last decades (the highest in Africa), and current chemotherapeutic drugs have not yielded the desired result, thus, the use of herbal plants for the treatment of diabetes is now being embraced globally as a better alternative to the prevailing medicinal approach, in which *Ruellia simplex* C. Wright (Acanthaceae) is one of such plants.

R. simplex is commonly called Mexican petunia or Texas petunia. It is a woody-based, rhizomatous annual plant that is grown as an ornamental herb. It originated in Mexico and has been distributed in many countries of west Africa such as Nigeria, Ghana, and the Ivory Coast (Iwu, 2014). The plants' branches from the ground into several woody-based stems with purple elongated leaves. Some species in the genus are suspected to be poisonous, especially those with reddish inflorescence or flowers. These species are often scarce and endemic to the Mediterranean regions extending sparsely into the tropics for example the *R. brittonianna* (Iwu, 2014).

Most species of the genus *Ruellia* have been used as antipyretic, antioxidant, analgesic, anti-spasmolytic, antiviral, antihypertensive, antifungal, antiulcer, antidiabetic, and anti-inflammatory agents in Nigeria. The plant *R. simplex* was reported to contain various secondary metabolites such as glycosides, alkaloids, flavonoids, and triterpenoids (Sharma et al., 2001).

The present study was carried out to evaluate the antinociceptive, anti-inflammatory, and antidiabetic potentials of an isolated novel fatty acid from the leaf methanol extract of *R. simplex*.

2. Materials and methods

2.1. Materials

2.1.1 Chmicals and reagents

Methanol, ethyl acetate, acetic acid, alloxan-monohydrate, carrageenan, and chloroform (analytical grades; JoeChem Nig. Ltd. for Sigma-Aldrich, St Lous Mo, USA), *n*-hexane (Benrock, Nig. Ltd.), distilled water, ibuprofen tablet (Alapharm, Nig. Ltd.), diclofenac sodium (Hovid, Nig. Ltd.), silica gel 60-120 mesh, silica gel 60 F254 TLC plates (Merk, Germany), LH-20 Sephadex (Sigma-Aldrich, St Lous Mo, USA).

2.1.2. Apparatus and instruments

Magnifying lens, NMR 850 MHz, LC-MS (Bruker, UK), GC-MS 790A (Agilent Technologies, UK), alpha II FTIR (UK), glass column (95 x 35 mm), vernier caliper, vacuum rotary evaporator (Buchi type, Shan, Model No: 130VRE, Haryana, India).

2.2. Methods

2.2.1. Collection and preparation of plant material

The fresh leaves of *R. simplex* were collected in the early morning hours between 5:30 am and 6:30 am in Eburu-Mmiri, Nsukka Enugu State, in March 2022, and authenticated by Mr. C. A. Ukwubile. A voucher specimen (No: FGO/002501) was deposited at the Archives of Medicinal Plants, Forest Guide, Ogurugu. The leaves were shade-dried for two weeks and pulverized into fine powders, weighed, and stored in a clean sample bottle before extracting with methanol for the extract.

2.2.2. Preparation of ethyl acetate fraction of R. simplex extract

Using liquid-liquid partitioning (LLP), 40 g of the obtained methanol extract was suspended in a separating funnel containing 700 ml of distilled water mixed with 300 ml of methanol. It was then successively partitioned with *n*-hexane (400 ml) (x 3), chloroform (400 ml) (x 3), and ethyl acetate (400 ml) (x 3). The fractions were dried using a rotary evaporator at 45 °C and separately weighed, kept in sample bottles, and stored in a refrigerator at 5 °C for subsequent use. Each fraction was tested for biological activities to obtain the most active fraction. The ethyl acetate fraction (EF) was the most bioactive fraction from the preliminary assays and was used in the current study.

2.2.3. Phytochemical screening

The EF was screened to determine the presence of some secondary metabolites which are likely to contain fatty acids like alkaloids, flavonoids, saponins, tannins, triterpenes, cardiac glycosides, carbohydrates, and fats/oils. The methods previously described by (Evans, 2002) were used for this purpose.

2.2.3.1. Isolation and characterization of novel fatty acid from the EF

The EF was subjected to silica gel (60-120 mesh size) open-column chromatography. Elution was carried out by gradient technique using *n*-hexane, chloroform, and acetone (2:4:4), respectively in the increasing order of their polarities. A total of twenty-five sub-fractions were collected and grouped into two based on their profiles on the TLC plate. Each sub-fraction was bio-monitored to select the most active sub-fraction. The most active sub-fraction

was subjected to comparative TLC using oleanolic acid as a marker compound for fatty acid. The separated compound showed similar color and Rf value with the authentic fatty acid used as standard. Finally, the isolated compound was checked for purity using the HPLC and then subjected to melting point determination, FT-IR, GC-MS, LC-MS, and NMR analysis to identify and elucidate the structure (Evans, 2002; Kumar et al., 2012).

2.2.3.2. Determination of melting point of the compound

The melting point of the isolated novel fatty acid was determined by using an IA9000 Series digital melting point apparatus (Electrothermal Engineering Ltd., UK). Briefly, 0.5 mg of the compound was introduced into a capillary tube and inserted into the heating bath of the melting point machine apparatus, and heated to determine the temperature after melting (Jothy et al., 2011).

2.2.3.3. FT-IR analysis of isolated compound

The fourier transform infrared (FT-IR) analysis of the compound was carried out using an Alpha II type FT-IR (Bruker, UK). The compound was scanned at 400-4000 cm^{-1} wavelength.

2.2.3.4. GC-MS analysis of isolated compound

GC-MS analysis of isolated compounds was carried out using Agilent 5977B GC/MSD with the following conditions: HP-5 capillary column (30 mm x 0.25 mm internal diameter., 0.25 µm thickness), oven temperature from 50 °C for 1 min, and increased to 250 °C at the value of 25 °C/min for 10 min, to a final temperature of 300 °C at the value of 20 °C/min. Head pressure was 15 psi, injector temperature, was 250 °C, injection mode 0.2 min splitless, injected volume 0.1 µl, the temperature of detector 270 °C, carrier gas helium. Mass spectra conditions: ion source temperature 225 °C, electron impact: 50 eV, acquisition mode scan (*m/z* 50–600) (Jothy et al., 2011).

2.2.3.5. LC-MS analysis of isolated compound

The liquid chromatography was performed using the quadrupole time-of-flight LC/-MS 6546 LC/Q-TOF (Agilent Technologies, UK) using the following experimental conditions: Zorbax SB C18, 2.1 x 50 mm column, 100% MilliQ water þ 0.1% formic acid 1.81 solvent A, 100% acetonitrile þ 0.1% formic acid solvent B, 0.3 ml/min flow rate, 3.1 µl injection volume, 30 min run time, and gradient elution technique was used (Siddiqui et al., 2011).

2.2.3.6. NMR analysis of isolated compound

The nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C NMR) was performed with a Bruker 850 MHz apparatus at the NMR laboratory of King Abdulaziz University, Jedda, Saudi Arabia. The isolated compound was dissolved in 2.5 µl deuterated chloroform ($CDCl_3$) and placed in the NMR tube. Then 0.1 µl of the solution was injected into the NMR port. The chemical shifts (δ) were reported as part per million (ppm) with reference to tetra-methyl-silane (TMS) as the internal standard solvent. The characteristics of the identified protons and carbons of the compound were compared with the proposed structure from the NIST library and other identification chemical libraries for organic compounds.

2.2.4. Antinociceptive activity of isolated novel fatty acid

To evaluate the antinociceptive activity, we investigated the peripheral and central antinociceptive activities of the compound. For peripheral antinociceptive activity, the acetic acid-induced writhing method in mice was used (Abotsi et al., 2017). Briefly, twenty mice of the opposite sex were grouped into four groups (five animals in each) and allowed to acclimatize in the laboratory for one week before evaluation. Group I is the negative control group which received 1% Tween-80 in normal saline solution, group II is the positive control group which received 50 mg/kg bw diclofenac sodium standard drug, while groups III and IV received 2 and 4 mg/kg bw doses of isolated compound intraperitoneal (i.p.), respectively before administration of 0.5% acetic acid (i.p.) 10 min later. After 15 min, the writhing of the abdomen was observed on each mouse every 5 min for 30 min using a magnifying lens. The analgesic activity was then expressed as follows:

$$Writhing\ inhibition\ (\%) = \frac{Wc - Ws}{\mathrm{Wc}} x\ 100$$

where Wc is the mean number of writhing in the control group and Ws is the mean number of the writhing of the treated groups.

On the other hand, the central antinociceptive activity was carried out using the tail immersion method, with morphine as the standard drug.

2.2.5. Anti-inflammatory activity evaluation

In determining the anti-inflammatory effect of the isolated fatty acid, carrageenan-induced paw edema of the rat model was used. Briefly, rats were grouped randomly into different groups. The plethysmometer was used to measure the rat's pedal volume to the ankle joint. Thereafter, the animals were injected with 100 µl normal saline,10 mg/kg bw of ibuprofen as well as 2 and 4 mg/kg bw of isolated fatty acid, respectively via intraperitoneal (i.p.) route 1 h before the injection of 50 µl of 1% carrageenan in the right hind paw of the rats (Abotsi et al., 2017). After the induction of edema in the rats, the volume of the paw was measured at intervals of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min. The edema volume was calculated using the formula below:

$$Change\ in\ paw\ edema\ volume = Final\ paw\ volume - \ Initial\ paw\ volume$$

2.2.6. Antidiabetic activity evaluation

The antidiabetic activity was evaluated using a glucose tolerance test (GTT), as was previously described by Mohamed et al. (2012) with moderate modification. Briefly, mice were first randomly selected and divided into four groups of three mice per group. Group I was administered 1% Tween-80 in normal saline (i.e., 0.1 ml/10 g bw) which served as the negative control group, and group II was administered 10 mg/kg bw Glibenclamide tablet (May & Baker Nig., Plc) standard drug, while groups III and IV were administered isolated fatty acid at doses of 5 and 10 mg/kg bw, respectively (i.p.). Then one hour later, all animals were administered i.p. in the penial vein with 1.5 ml 1% alloxan monohydrate (Sigma-Aldrich St. Lous Mo, USA). Six hours after alloxan monohydrate administration (i.p.), blood samples were then collected from each animal by the tail tips in 30, 60, 120, 180, and 240 min. Finally, the blood glucose levels were determined using a glucometer (ACCU-CHEK Softclix, Roche), after noting the glucose baseline in each mouse of each group.

2.3. Statistical analysis

The data generated from the studies were subjected to a one-way analysis of variance (One-way ANOVA) using SPSS statistical software (version 22). Values are expressed as mean ± SD for various groups. The values of $p < 0.05$ were taken as statistically significant.

3. Results and discussion

3.1. Preliminary phytochemical screening

In the current study, preliminary phytochemical screening of the leaf extract revealed the presence of alkaloids, tannins, saponins, flavonoids, and triterpenes as well cardiac glycosides (Table 1).

Table 1. Phytochemical contents of *R. simplex* leaf methanol extract

Constituents	Interference
Carbohydrate	+
Saponins	+
Alkaloids	++
Flavonoids	++
Tannins	+
Triterpenes	+
Phytosterols	++
Cardiac glycosides	+
Fats/oils	++
Proteins	-
Anthracene	-

+: Moderately detected, ++: Largely detected, -: Not detected

3.2. Structural elucidation of isolated fatty acid

The isolated fatty acid was an amorphous yellow compound with a fine smell like groundnut oil typical of fatty acid. The structure of the isolated fatty acid was elucidated using various techniques previously described. FTIR showed a non-bonded OH group (3000 cm^{-1}), symmetrical stretching of OH groups (2984 and 2500 cm^{-1}) of fatty acids, an α-unsaturated carboxylic acid group (2000 cm^{-1}), and mono-substituted aromatic rings (1500 and 1000 cm^{-1}) stretching, which are an indication of fatty acids (Kumar et al., 2017). The MS data: EIMS $[M]^+$ at *m/z* 467, while the LC-MS data showed pseudomolecular ions $[M+H]^-$ at 465.1 and $[M+H]^+$ at 617.1 *m/z*; RT, 2.756 min. ^{1}H-NMR spectrum analysis revealed a signal δ 0.772 ppm (H-1, d, 0.777) of methylene carbon C-2, characteristic of fatty acids. The proton H-13 at δ 1.468 ppm (s) indicated an olefinic ring of unsaturated fatty acids The results obtained were represented in Figure 1, Table 2, Figure 2, Figure 3, Figure 4, and supplementary files (S1-S5).

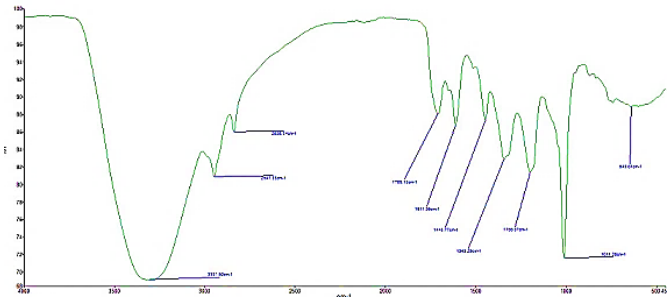

Figure 1. FTIR spectrum of isolated novel fatty acid

Table 2. ^{1}H and ^{13}C (850 MHz) NMR data of isolated novel compound in CDCL3

Peak No.	δ_H ppm (850 MHz)	δ_C ppm (850 MHz)
2	0.772 (d, 0.777)	33.08
3	0.885 (m)	32.91
4	0.902 (s)	32.92
5	0.918 (d, 0.925)	32.62
6	0.968 (q)	32.43
7	0.984 (s)	31.95
8	1.079 (s)	31.45
9	1.132 (s)	31.01
10	1.281 (m)	30.69
11	1.330 (s)	30.57
12	1.453 (q)	30.19
13	1.468 (s)	29.73, 29.69
14	1.733 (m)	29.40
15	1.782 (s)	29.28, 28.14, 28.10, 27.98, 27.67, 27.16, 27.12, 26.99, 26.48, 25.94, 24.12, 23.62, 23.59, 23.40, 23.29, 22.94, 22.73

3.3. Antinociceptive and anti-inflammatory effects of novel fatty acid (2,4-PHPBEa)

The result obtained showed that the compound *2,4-PHPBEa* significantly reduced the withing of abdominal pains in the rats in a dose-dependent fashion from 2 to 4 mg/kg bw. The novel fatty acid from *R. simplex* greatly inhibited the peripheral pains induced by acetic acid in 30 min, as the doses increased from 2 to 4 mg/kg with 66.19% and 82.71% inhibition of writhing, respectively (Table 3). These results were statistically significant ($p < 0.05$; one-way ANOVA) when compared to the standard drug diclofenac sodium. On the other hand, the central antinociceptive activity (Table 4) of the novel fatty acid at the same doses showed significant dose-dependent decreases in response to tail flicking or licking after 120 min administration when compared to the standard drug morphine ($p < 0.05$).

3.4. Antidiabetic effect of novel fatty acid 2,4-PHPBEa

There was a dose-dependent reduction in the level of plasma glucose in the alloxan-induced diabetic rats from 30 to 240 min (Figure 7). However, the standard drug did not show a significant decrease in hyperglycemic effect within this period ($p < 0.05$).

Fatty acids are a group of organic compounds which are structurally made up of long chains of carbons terminating two functional groups of a carboxylic acid and a methyl group on different sides. Naturally occurring fatty acids often possess mostly even number of carbon atoms, which are saturated or unsaturated depending on the bonds existing between the carbon atoms. Medicinal plants and vegetable oils contain palmitic acid, stearic acid, oleic acid, and linoleic acid. These fatty acids are saturated and unsaturated in nature consisting of single and double bonds respectively (Pinto et al., 2017). These fatty acids have been isolated from various plant families where they are used for treating many diseases such as pain, inflammation, diabetes, cancers, hemorrhoids, etc. Some of these fatty acids have been used for the formulation of various dosage forms by pharmaceutical industries for therapeutic purposes.

An evaluation of the phytochemical contents of plants (Table 1), is important to elucidate the various classes of secondary metabolites that are present in the plant. This is very crucial in that secondary metabolites such as alkaloids, saponins, and flavonoids, for example, have been used as anticancer, anti-hemorrhoid, and antioxidant agents, respectively (González et al., 2011). It has been reported that a plant's therapeutic activities are also solely dependent on the

type of metabolites it contained at a given period (Valdés et al., 2020). These phytochemicals play crucial roles in ameliorating various human diseases in traditional medicine. For instance, saponin and flavonoid groups of phytochemicals possessed compounds that are used as analgesics and anti-inflammatory agents, while alkaloids and triterpenes showed both anticancer and antimalarial properties, respectively. Tannins, on the other hand, also possess an anti-diabetic effect, whereas cardiac glycosides help to stimulate the cardiac muscles in hypertension conditions (Jacobs et al., 1990). The roles played by these metabolites were not at variance from their roles in this study. In the fractionation and isolation of novel fatty acid, a total of twenty-five fractions were collected and grouped into four (I, II, III, IV) based on their TLC profiles, and were bio-monitored for antinociceptive, anti-inflammatory, and antidiabetic effects. Group II (i.e., fractions 9-15) showed the biologically most potent activity. Further fractionation of group II using normal phase short column silica gel chromatography eluted by gradient elution using *n*-hexane:chloroform (1:2), and *n*-hexane:ethyl acetate (2:8) yielded 2.8 mg compound A and 28 mg compound B, respectively. Compound B showed 75% (antinociceptive), 86.4% (anti-inflammatory), and 78.12% (antidiabetic) effects, while compound A produced less than 2% biological activities within 24 h testing.

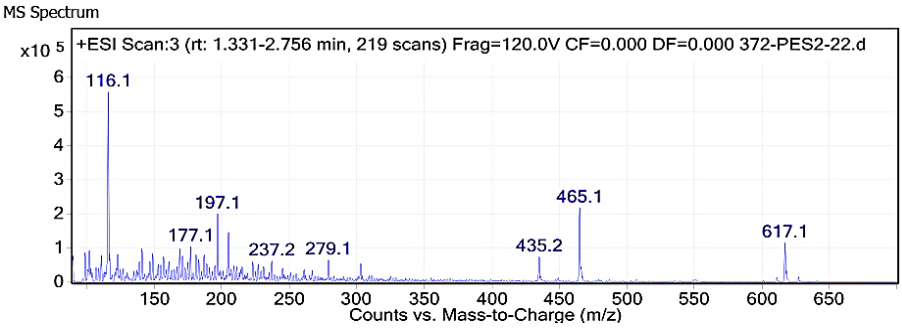

Figure 2. LC-MS of isolated novel fatty acid

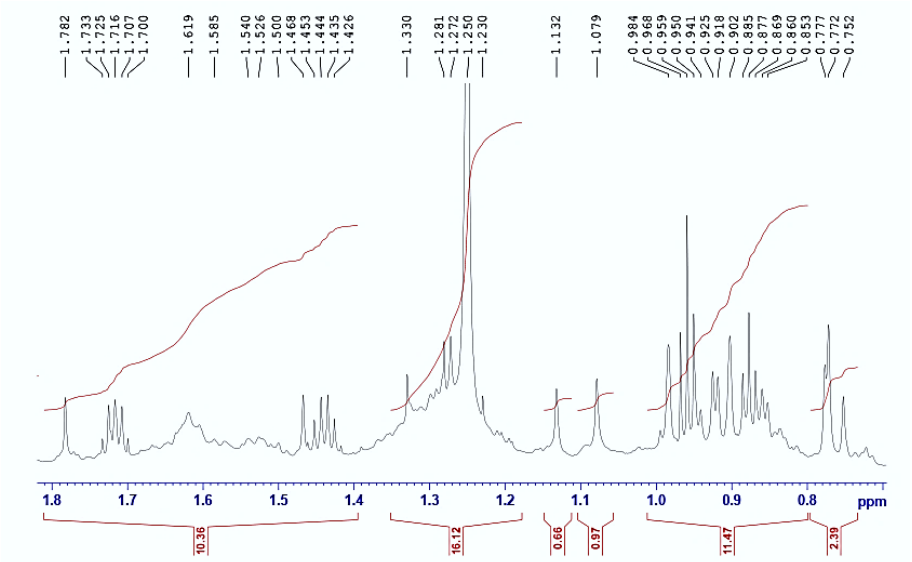

Figure 3. ^{1}H-NMR spectrum of isolated novel fatty acid

The isolated novel compound in the current study possessed the following properties: physical properties; yellow amorphous in appearance (28 mg), melting point; 164.4 °C, the UV data: [MeOH] λmax 217.8 and 308.3 nm, [MeOH+NaOH] λmax 227.5 and 406.2 nm (Figure S1a, b). The FTIR spectrum showed the presence of a non-bonded OH group (3000 cm^{-1}), symmetrical stretching of OH groups (2984 and 2500 cm^{-1}) of fatty acids, an α-unsaturated carboxylic acid group (2000 cm^{-1}), and mono-substituted aromatic rings (1500 and 1000 cm^{-1}) stretching (Figure 1), which are indicative of the presence of fatty acids (Kumar et al., 2017). The MS data showed EIMS $[M]^+$ at *m/z* 467 with molecular formula; $C_{28}H_{26}O_6$ (Figure S2), while the LC-MS data (Figure 2) showed pseudomolecular ions $[M+H]^-$ at 465.1 and $[M+H]^+$ at 617.1 *m/z*; RT, 2.756 min. The NMR analysis of the compound revealed the following: ^{1}H-NMR spectrum (Table 2, Figure 3) analysis revealed a signal δ 0.772 ppm (H-1, d, 0.777) of methylene carbon C-2, characteristic of fatty acids. The proton H-13 at δ 1.468 ppm (s) indicated an olefinic ring of unsaturated fatty acids while the proton H-15 at δ 1.782 ppm (s) contained OH groups linked to a carbonyl carbon which are typical of fatty acids. The ^{1}H-^{1}H COSY correlations of (Figure S3) showed that the proton H-10 (δ 0.82 ppm) correlates with H-3 (δ 0.79 ppm), H-7 (δ 0.89 ppm), and H-9 (δ 1.09 ppm); proton H-12 (δ 0.89 ppm) correlates with H6, and H-15 (δ 1.42 ppm) correlates with H-12 and H-13. These patterns of correlations indicated various linkages between aromatic rings of benzene. The HMBC correlations (Figure S4) showed that the proton H-9 was double bonds connected with C-7 and C-16, H-11 was connected by double bonds to C-18 and C-20, while the proton at H-12 was double bonds connected to the carbonyl carbon C-26 of carboxylic acid. The HSQC spectrum (Figure S5) did not show multiple bond corrections and was typical of fatty acids. The ^{13}C-NMR spectrum of the compound (Table 2, Figure 4) showed mainly methylene carbons

with few methine carbons at δ 33.08 to 22.73 ppm. In all, the ^{13}C-NMR spectrum indicated twenty-eight (28) carbon atoms with no quaternary and methane carbon signals. When these NMR spectral data were compared with chemical libraries, the NIST library, and published literature, there was no match to the isolated fatty acid found, hence, it was concluded that the compound was novel. However, following IUPAC rules for naming of organic compounds, the fatty acid was identified as (2*E*,4*E*)-4-(4'-(2-phenyl-4,4a,6,8a-tetrahydropyrano[3,2-d] [1,3]dioxin-6-yl)-[1,1'-biphenyl]-3(4H)-ylidene) but-2-enoic acid (Figure 5); *m/z*: 470.17 (100.0%); exact mass: 470.17; elemental analysis: C (74.03), O (20.40), H (5.57). The addition of three extra atoms as opposed to that of the MS data (467) could be due to the overlapping of some peaks in the MS or the resolution of some other elements such as esters that are naturally attached to most unsaturated fatty acids (Jacobs et al., 1990). Our study identified the novel compound as a type of fatty acid because of the information derived from the spectroscopic data described above.

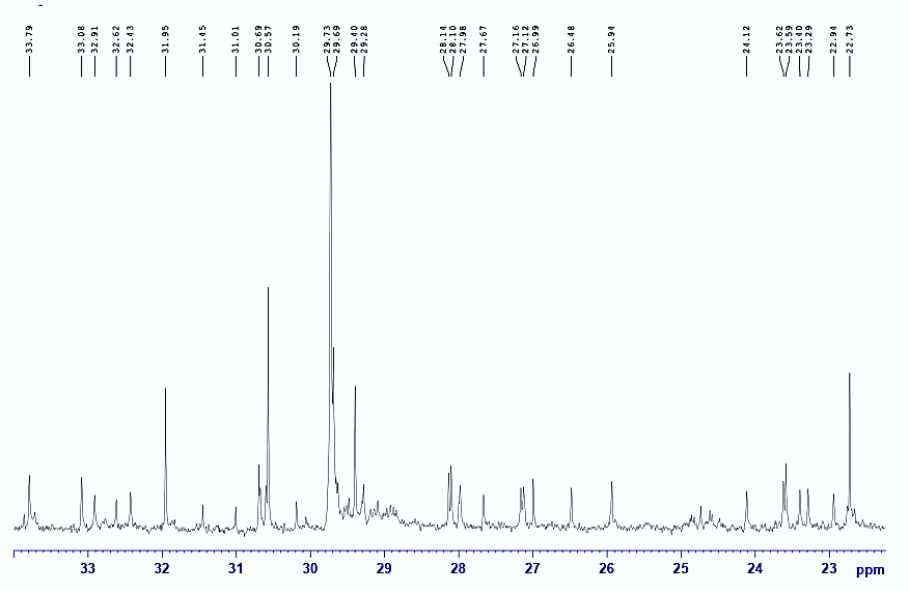

Figure 4. ^{13}C-NMR spectrum of isolated novel fatty acid

It has been previously reported that fatty acids such as linoleic, arachidic, and palmitic acids showed analgesic and anti-inflammatory potentials in inflammation-induced rat models (Forman et al., 2006). This report was not different from that obtained in the current study, where the isolated fatty acid showed dose-dependent antinociceptive and anti-inflammatory effects at higher doses. The isolated novel fatty acid showed significant inhibition in abdominal writhing in the animals within the experimental periods. The presence of olefinic rings and repeated chains of oxygen-bonded aromatic rings could be responsible for the biological activities of this compound. Again, many fatty acids originate from secondary metabolites, such as flavonoids, were reported to have anti-inflammatory potential. This is because they inhibit the production of mediators of inflammation by altering pathways like arachidonic acid, thereby, inhibiting many enzymes like prostaglandin, ATPase, lipoxygenase, cyclooxygenase, NADH oxidase, phospholipases, protein kinase, peroxidases, tyrosinases, and hydrolases. Thus, this novel fatty acid must have acted in a similar way to achieve the obtained result in the current study (Padmanabhan & Jangle, 2012). The number of abdominal writhing displayed by the compound showed its antinociceptive potential which was achieved through the prevention of prostaglandin production, that is peripheral pain inhibition mechanism investigated in this study. Therefore, the novel fatty acid must have achieved this biological activity by modulation of the peripheral and central routes of impulse (De Farias Freire et al., 1993; Pan et al., 2010).

Table 3. Peripheral antinociceptive activity of novel *2,4-PHPBEa* in acetic acid induced rats

Group	Number of writhing (± SD)	Inhibition of writhing (%)
Normal saline	18.04 ± 1.02	-
Standard drug	10.21 ± 0.24	43.40
2 mg/kg	6.10 ± 0.01*	66.19*
4 mg/kg	3.12 ± 0.02*	82.71*

Values are mean ± SD (n = 5)
*Statistically significant at $p < 0.05$ compared with the standard drug diclofenac sodium (one-way ANOVA followed by Duncan's multiple range test).

Similarly, inflammation is usually associated with signs like edema, redness, pain, as well as dysfunction of organs and organs tissues, and it is also associated with secretions of pain mediators (Vane & Botting, 1998). In the current study, at the doses of 2 and 4 mg/kg bw of the novel fatty acid, the diameters of carrageenan-induced paw edema decreased progressively within 60 min (Figure 6). The compound was able to achieve this by inhibiting the release of numerous mediators of inflammation like interleukin-6, nitric oxide, and α-tumor necrosis factor, which are body's innate immune mediators and prostaglandins (PGE2) (Dhasmana et al., 2014). The ability of *2,4-PHPBEa* to prohibit the production of these promoters of inflammation makes it an excellent agent of inflammation which was capable of preventing the expression of these pro-inflammatory factors. The result obtained was statistically significant when compared with the standard drug ibuprofen ($p < 0.05$).

Table 4. Central antinociceptive activity of novel fatty *2,4-PHPBEa* by tail immersion

Group	Number of responses (in 2 hours)			
	30 min	60 min	90 min	120 min
Normal saline	15.01 ± 1.11	14.06 ± 1.10	8.00 ± 0.11	7.01 ± 0.01
Standard drug	12.22 ± 1.02	12.00 ± 0.20	12.24 ± 1.21*	8.04 ± 0.28*
2 mg/kg	8.22 ± 1.21	8.00 ± 0.10	7.24 ± 0.12	7.10 ± 0.01*
4 mg/kg	4.08 ± 0.01	3.14 ± 0.01	3.01 ± 0.02	2.14 ± 0.01*

Values are mean ± SD (n = 5).
*Statistically significant at $p < 0.05$ compared with the standard drug morphine (one-way ANOVA followed by Duncan's multiple range test).

One of the largest worldwide health challenges currently is diabetes which has resulted in various types of life-threatening sicknesses like stroke, hypertension, and cardiac myopathy (Maritim et al., 2003). Since there are no guaranteed drugs currently to effectively manage and treat diabetes, there is a need to search for novel compounds with significant antidiabetic effects to serve as preferred drugs to the existing ones. In this present study, the result showed that there was a significant difference in plasma glucose baseline levels in all the groups before oral administration of the control standard drug Glibenclamide and various doses of *2,4-PHPBEa* (2 and 4 mg/kg bw). The ability of this compound to significantly reduce the glucose level in the animals was due to inhibition of the activity of α-glucosidase which facilitates the production of excess insulin (Wiernsperger, 2003). Some fatty acids have also been reported to be effective in ameliorating diabetic conditions, thus, the current novel fatty acid should also belong to this class of compounds.

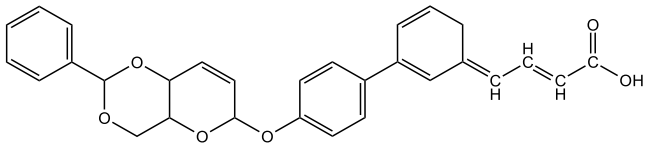

Figure 5. Proposed structure of novel fatty acid
(2*E*,4*E*)-4-(4'-(2-phenyl-4,4a,6,8a-tetrahydropyrano[3,2-d][1,3]dioxin-6-yl)-[1,1'-biphenyl]-3(4H)-ylidene)but-2-enoic acid
($C_{28}H_{26}O_6$), *m/z*: 470.17 (100.0%); exact mass: 470.17; elemental analysis: C (74.03), O (20.40), H (5.57).
The fatty acid was abbreviated as *2,4-PHPBEa*.

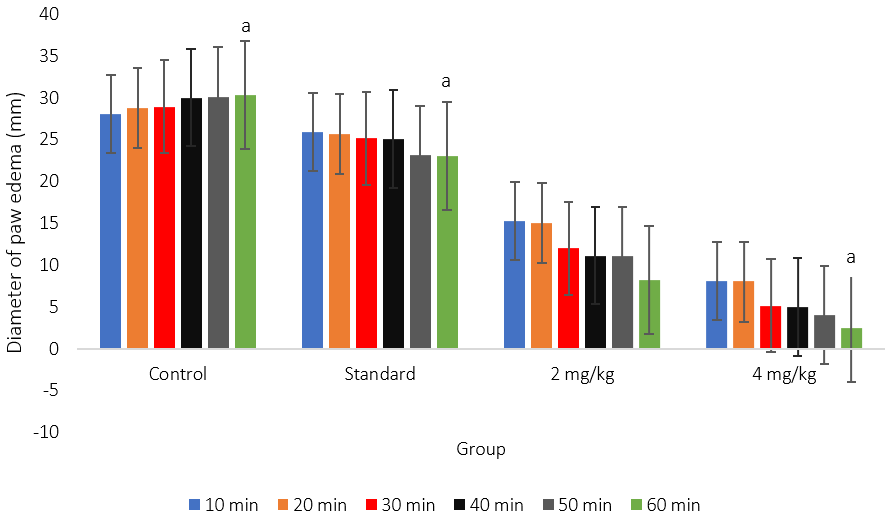

Figure 6. Effects of novel isolated fatty acid *2,4-PHPBEa* on diameter of carrageenan-induced paw edema in rats
Results are mean ± SD (n = 5). 'a' means statistically significant vs control ($p < 0.05$; one-way ANOVA)

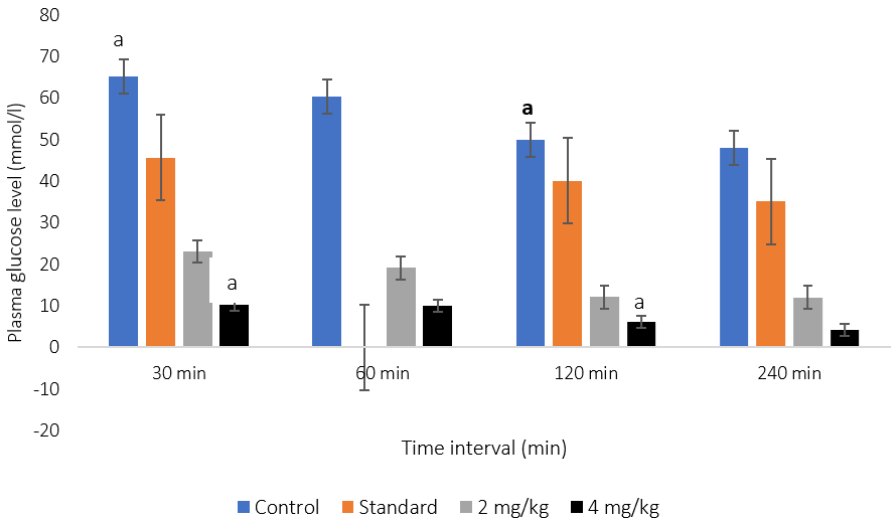

Figure 7. Effects of novel isolated fatty acid *2,4-PHPBEa* on plasma glucose level of alloxan-induced diabetic rats.
Results are mean ± SD (n = 5). 'a' means statistically significant vs control ($p < 0.05$; one-way ANOVA)

4. Conclusions

Our study showed that the novel fatty acid *2,4-PHPBEa* possessed potential antinociceptive, anti-inflammatory, and antidiabetic activities, in correlation with the inhibition of various promoters of pains, inflammation, and diabetes by blocking their metabolic pathways responsible for the negative effects on health. Furthermore, the current study design covered structural elucidation procedures to characterize the novel compound, which finally affirmed it as an unsaturated fatty acid. The ability of the characterized novel compound to act significantly on pain, inflammation (NSAIDs-like), and diabetes showed that it could be the promising'hit' bioactive compound in *R. simplex* which may be used against these illnesses, and further justifies its ethnomedicinal use in folkloric medicine. Finally, it is suggested that the mechanism of action of this novel fatty acid in pain, inflammation, and diabetes should be investigated for future research.

Acknowledgments

The authors were grateful to Dr. Mary Chama from the Department of Chemistry, University of Ghana, Legon, Ghana for helping out in some of the spectroscopic studies as well as Prof. Regina Appiah-Opong from the Department of Clinical Pathology, Noguchi, Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

Ethical approval for this study was obtained from the Research Ethical Committee of the University of Jos, Nigeria with approval number of UJ/FPS/F17-00379.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Funding

None.

CRediT authorship contribution statement

Cletus Anes Ukwubile: Conceptualization, Investigation, Methodology, Final analysis, Writing -original and final draft
Henry Nettey: Resources, Formal analysis, Supervision
Troy Salvia Malgwi: Formal analysis, Investigation, Methodology, Software
Nnamdi David Menkiti: Formal analysis, Investigation, Software

ORCID Numbers of the Authors

C. A. Ukwubile: 0000-0001-7183-4510
H. Nettey: 0000-0002-5408-5957
T. S. Malgwi: 0000-0001-5051-8754
N. D. Menkiti: 0000-0001-6979-0088

Supplementary File

The supplementary file accompanying this article is available at https://ijpbp.com/index.php/ijpbp/libraryFiles/downloadPublic/14.

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