Nigerian Journal of Pharmaceutical Sciences
Vol. 23, No1, 2024, ISSN: 0189-823X
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ANTIOXIDANT AND *IN VITRO* β-HEMATIN INHIBITION POTENTIALS OF THE n-HEXANE FRACTION AND ITS ISOLATED COMPOUND FROM THE AERIAL PARTS OF *VERNONIA CINEREA* LESS. (ASTERACEAE)

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ABSTRACT

Vernonia cinerea, a plant belonging to the Asteraceae family. The plant is traditionally used in ethno-medicine for the treatment of various diseases, including malaria. Antioxidant use in malaria infection confers protection to the erythrocytes membrane of the host and prevents parasite damage to intact red blood cells. Heme accumulates when the plasmodium utilizes hemoglobin of the host and, an antimalarial agent with the ability to inhibit the conversion of heme to hemozoin is regarded as an invaluable target to fight the infection with β-hematin inhibition as an excellent tool for the evaluation of herbal extract. This study intends to isolate and assess the antioxidant and β-hematin inhibition activities of the n-hexane fraction (HF) and its isolated compound from the methanol extract of the plant's aerial parts using standard protocols. Column chromatography was used to purify the HF and, then analyzed using Fournier transform infra-red (FTIR) and nuclear magnetic resonance (NMR) spectroscopies for structural determination. This led to the isolation of a compound coded as VC3, and NMR spectroscopy of the isolated compound showed it to be a mixture of triterpenoid. Result of radical scavenging activity showed ascorbic acid to be more than twice the effect in comparison to HF and VC3. However, the result of β-hematin inhibition of the isolated compound was higher than the positive standard as well as HF. The isolated compound showed slight antioxidant and appreciable β-hematin inhibition effects.

Keywords: Antioxidant, β-hematin, Column, Fraction, Hemoglobin, Vernonia

INTRODUCTION

Medicinal plants (MPs) are made up of different groups of active metabolites (Simplice *et al*., 2022), such as alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, and tannins (Abubakar and Haque, 2020), each with unique pharmacological potentials (Vivek *et al*., 2016). Pure compound with known chemical structure is obtained through bioassay guided fractionation of plant extract, which begin with testing for the biological activity. Once the extract tested, is found to be pharmacologically active, next is to proceed with the fractionation. Various fractions obtained are tested for their biological activities. The most active fraction is then taken for isolation. Lastly, the isolated compound is identified and also tested for biological activity (Abubakar and Haque, 2020).

Column chromatography (CC) plays an important role in natural product chemistry and the discovery of new pharmaceutical compound(s). This technique involves preparation of the sample, packing and pouring of the sample into the column,

elution of fractions, and monitoring of each fraction using thin layer chromatography, this is closely followed by fournier transform infrared (FTIR) nuclear magnetic resonance (NMR) spectroscopies to determine the structure of isolated compound (Vivek *et al*., 2016).

Fournier transform infrared (FTIR) spectroscopy is a technique that assess functional groups in a compound by defining the physical and chemical properties (Nandiyanto *et al*., 2019). It also, highlight presence of single, double, and multiple bonds in a compound (Abubakar and Haque, 2020). Nuclear magnetic resonance (NMR) on the other hand is a technique that pays more attention to the physical characters of the bioactive molecule such as number and array of the carbon atom, presence of isotopes of carbon, hydrogen atom, and protons. It also described how atoms are arranged in a molecule (Abubakar and Haque, 2020).

Oxidation is a critical metabolic process through which the body can perform living functions properly (Hoang *et al*., 2018). However, oxidative metabolism produces free radicals especially during malaria infection (Vasquez and Rodriguez, 2021), where large amount of hemoglobin is degraded to generate large amounts of the reactive oxygen species (Adesegun *et al*., 2017; Bahaah *et al*., 2019) and, when highly accumulated, result in oxidative stress (OS) that causes damage to cell structures (Al-Trad *et al*., 2018) of the host. Although the body has its own systems to balance the ratio between oxidants and antioxidants, it can get overwhelmed sometimes like every other machinery (Guchu *et al*., 2020). Many synthetic antioxidants abound, but their use have been suspected to cause or promote negative health effects, hence stronger restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants (Chakraborty *et al*., 2018).

Antioxidants derived from plants are highly recommended because, they are safer and can block oxidative damage through the reduction of free radicals in the body (Marjoni and Zulfisa, 2017). Medicinal plants have always been considered a source of natural antioxidants, which are highly effective in preventing the processes of oxidation by neutralizing free radicals (Ramaite *et al*., 2020). 2,2-diphenylpicryl-1-hydrazyl (DPPH) is a stable organic free radical with an absorption band at 517 nm, it loses this absorption when it accepts a proton or a free radical species, resulting in a visually noticeable discolouration from purple to yellow product; diphenylpicryl hydrazine (Kankara and Go, 2016), the intensity of this yellow colour depend on the number of protons donated by the plant extract, fraction or the isolated compound. This free radical is capable of incorporating many samples in a short period and distinguishes active ingredients at low concentrations (Arash *et al*., 2015).

Malaria is a life-threatening disease caused by the reproduction of *Plasmodium falciparum* in the host erythrocyte (Babatunde and Wande, 2017), the parasite utilizes hemoglobin as its source of nutrients for growth and proliferation, however, the heme released during the break down of haemoglobin accumulates to the detriment of parasite (Akkawi *et al*., 2014), for the plasmodium to survive, it has to detoxify free heme via biocrystalization into hemozin (malaria pigment) which is non-toxic (Abiodun, 2018), hence, inhibition of hemozoin formation using antimalarial compounds is regarded as an invaluable target to combat the malaria infection (Afshar *et al*., 2018). Notable phytochemical compounds such as sesquiterpenes, diterpenes, steroids, flavonoids, alkaloids,

stilbenes and coumarin derivatives have displayed antimalarial properties in various *in vitro* tests (Afshar *et al*., 2016).

Antimalarial drugs such as quinine and artemisinin are from natural sources, they are available and used to inhibit hemozoin formation (Babatunde and Wande, 2017), however, there is a need to screen more plants to discover novel natural drugs especially those with phytotherapy potentials for the management of malaria (Akkawi *et al*., 2014) as well as to tackle the issue of *Plasmodium falciparum* resistance to the existing antimalarial agents (Wubayehu and Melshew, 2019) and, to also predict how the fraction and the isolated compound act.

This research work therefore, intends to isolate and characterize a compound in the n-hexane fraction (HF) from methanol extract of the aerial parts of *Vernonia cinerea*, using column chromatography and spectroscopic analysis (fournier transform infra-red and nuclear magnetic resonance), and further evaluate the antioxidant and β-hematin inhibition effects of the fraction and its isolated compound to get a bioactive entity with dual effects following standard procedures.

MATERIALS AND METHODS

Materials

Ascorbic acid, Glass column of dimension 75 by 3.0 cm with a capacity of 500 mL, silica gel size 60 - 120 µm, n-Hexane, ethyl acetate, glass rod, cotton wool, pre-coated silica gel thin layer chromatography plate, *p*-anisaldehyde, Libermann- Burchard, Ferric chloride, Aluminium chloride, Bontragers and Dragendorf, Electrothermal MP apparatus- England, capillary tubes, β-hematin solution, chloroquine, sodium acetate, glacial acetic acid, centrifuge, dimethyl sulphoxide (DMSO), NaOH solution and spectrophotometric machine (Abubakar and Haque, 2020).

Methods

Column chromatography of n-hexane fraction from methanol extract of aerial parts of *Vernonia cinerea*

A glass column of dimension 75 by 3.0 cm with a capacity of 500 mL was wetted with n-Hexane solvent, plugged with cotton wool from the bottom and packed with 80 g silica gel, size 60 - 120 µm already mixed with 200 mL n-Hexane, the side of the column was tapped gently with a glass rod to displace air bubbles and ensure even compaction of particles. Three (3) g of the HF was mixed with 20 g of silica gel, dried for 3 hours and introduced into the column, the top was plugged with cotton wool, and more solvent was added into the column, covered with foil paper and allowed to stand overnight to ensure stabilization before elution began. The column was eluted continuously in a gradient profile with hexane 100%, hexane: ethyl acetate mixtures in the ratio of increasing polarity and ethyl acetate 100 %. Column fractions were collected in 20 mL aliquots and monitored on a TLC pre-coated silica gel plate, air dried and sprayed with *p*-anisaldehyde, this was further air dried and heated at 110 °C for visualization of spots.

Purification of column fractions

Column fractions 42-55 were pooled together, eluted further with hexane: ethyl acetate (9:1), and the fractions collected were monitored on TLC plates using hexane: ethyl acetate (8:2; 7:3). Similar column fractions showing distinct single spots were pooled together, dried, coded as VC3 and properly stored in an airtight container for further use.

Physicochemical evaluation of isolated compound

Physical Characters of VC3
These include colour and solubility in different solvents (hexane, chloroform, ethyl acetate and methanol).

TLC of VC3 Sprayed with Specific Reagents
TLC plates were sprayed with specific detecting reagents to identify specific phytochemicals: Libermann- Burchard for detecting the presence of terpenes, Ferric chloride for phenolic compounds, Aluminum chloride for flavonoids, Bontragers for anthraquinones and Dragendorf reagents for alkaloids.

Determination of the melting point of VC3
The melting point (MP) was determined using the Electrothermal MP apparatus-England. Three capillary tubes were sealed from the bottom and the free ends dipped into the

sample; they were placed individually in a hollow plastic (30 cm long) severally to compact the sample to the bottom of the tubes. These tubes were arranged in the MP apparatus, a thermometer was placed in position and then connected to the main with an initial temperature of 5 ^{0}C.

Structural elucidation of VC3
For the structural elucidation, 10 and 50 mg of isolated compound (VC3) were taken to the Multi-user Science Research Laboratory, A.B.U, Zaria for Fournier Transform Infra-red (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopic analysis. The NMR spectra were obtained at 400 MHz NMR spectrometer (Bruker, Germany) for 1H-NMR and 13C-NMR.

Antioxidant activities of HF and isolated compound using 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging method
The free radical scavenging activity of the HF and VC3 was measured in terms of hydrogen donating ability using the stable free radical; DPPH. Briefly, 0.1 mM solution of DPPH in methanol was prepared, and 1 mL of this solution was added to 3 mL of the test samples (HF and VC3) and ascorbic acid (positive control) solutions at different concentrations (31.25, 62.5, 125, 250 and 500 µg/mL) respectively. Methanol was used as negative control (3 mL) and 1 mL of DPPH solution was also added. These mixtures were vortexed thoroughly and allowed to stand at room temperature for 30 minutes, after which, their absorbances were measured at 517 nm using a spectrophotometer (Spectrum Lab China). Lower absorbance values of the reaction mixture indicate higher free radical scavenging activity (Aadesariya *et al*., 2017). The ability of HF and VC3 to scavenge the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{AO-AI}{AO} \text{ X } 100$$

Where, AO is the absorbance of the negative (methanol) control reaction, and AI is the absorbance in the presence of HF, VC3 and positive control reactions. All the tests were performed in triplicates and an average was taken.

Evaluation of *in vitro* β-hematin inhibition of HF and isolated compound
The procedures of this assay were carried out on HF, VC3 and chloroquine. Exactly 500 µL of HF, VC3 and chloroquine each of varying concentrations; 0.5, 1, 2, and 4 mg/mL were incubated with 500 µL of hematin solution for 20 minutes at 37 ^{0}C, this was followed by the addition of 1000

µL of sodium acetate and 250 µL of 17.4 M glacial acetic acid at the pH 4.8 essential for β-haematin formation, the reactions were allowed to continue for 1 hour at 70 ^{0}C for complete polymerization, the resulting solutions were centrifuged at 10,000 rpm for 5 minutes and the supernatants discarded. The pellets were washed three (3) times with 2,000 µL of 100 % dimethyl sulphoxide (DMSO) until clear supernatants were obtained. The pellets were then dissolved in 2,000 µL of 0.1 M NaOH and the absorbances were measured at a wavelength of 630 nm (Akkawi *et al*., 2014; Delazara *et al*., 2018). Each tube was prepared in triplicate. The results were recorded as % inhibition of β-hematin synthesis compared to positive control using the following formula:

$$\% \text{ Inhibition} = \frac{AO - AA}{AO} \text{ X } 100$$

where AO is the absorbance of negative control and AA is the absorbance for HF, VC3 and positive control (chloroquine) respectively.

Statistical Analysis

Results were presented as figures and tables where applicable. Data generated were expressed as mean ± Standard Error of Mean (± SEM) using analysis of variance (ANOVA) for antioxidant study as well as *in vitro* β-hematin inhibition activity.

RESULTS

Column chromatography of HF

The Column chromatography of HF led to a total of 88 collections in 20 mL aliquots each, these were monitored on TLC plates and fractions that gave similar TLC profiles were pooled together, these column fractions and the solvent systems in which they were developed were presented in Plates I, II and III, and Table 3.1. Furthermore, the column fractions were pooled together based on physical appearance of the TLC profile into eight different pools, developed in hexane: ethyl acetate (9:1) (Plate IV and Table 3.2) together with HF. At the purification column, 29 column fractions were collected and fractions 12 to 14 showed single distinct spots (Plate V), these were pooled together and coded as VC3.

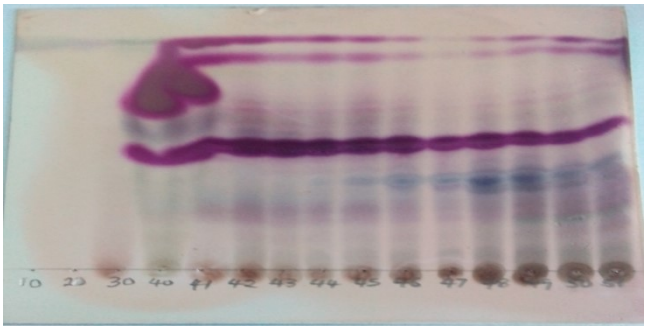

Plate I: Column Fractions of n-Hexane Fraction from Methanol Extract of *Vernonia cinerea* (10, 20, 30 and 40 are Pooled Column Fractions While 41 to 51 are Individual Fractions) Developed in Hexane: Ethyl acetate (8:2) and sprayed with *p*-anisaldehyde

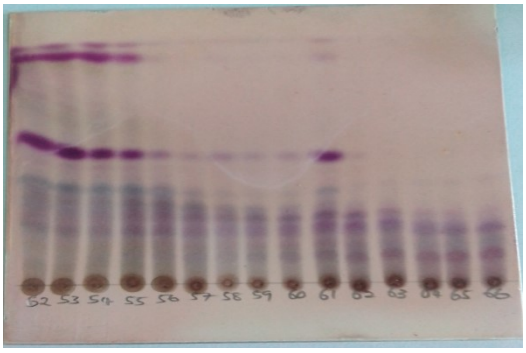

Plate II: Column Fractions 52 to 66 of n-Hexane Fraction from Methanol Extract of *Vernonia cinerea* Developed in Hexane: Ethyl acetate (8:2) and sprayed with *p*-anisaldehyde

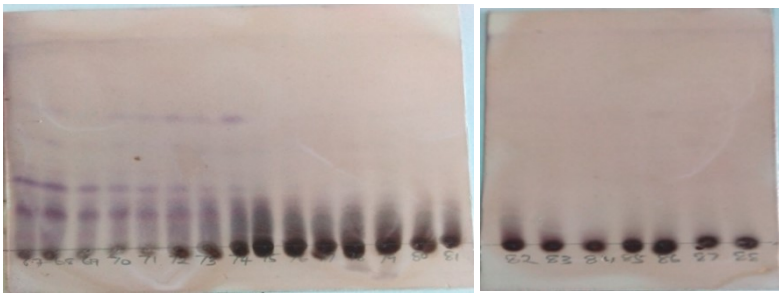

Plate III: Column Fractions 67 to 88 of n-Hexane Fraction from Methanol Extract of *Vernonia cinerea* Developed in Hexane: Ethyl acetate (8:2) and sprayed with *p*-anisaldehyde

Table 1: Column Chromatographic Separation of n-Hexane Fraction

Fraction Collections	Solvent System	Colour of Spot	No. of Spots
1 to 10	100 % Hexane	Colourless	Nil
11 to 20	H: EA (95:5 mL)	Colourless	Nil
21 to 30	H: EA (90:10 mL)	Colourless	Nil
31 to 40	H: EA (85:15 mL)	Deep purple, ash	3
41 to 66	H: EA (80:20 mL)	Deep purple, brown, faint green, blue	≥ 5 to ≤ 20
67 to 71	H: EA (70:30 mL)	Purple, brown	5
72 to 74	100 % EA	Purple, brown	5
75 to 88	100 % EA	Brown	No clear spots

Note: H: EA = n-Hexane: Ethyl acetate

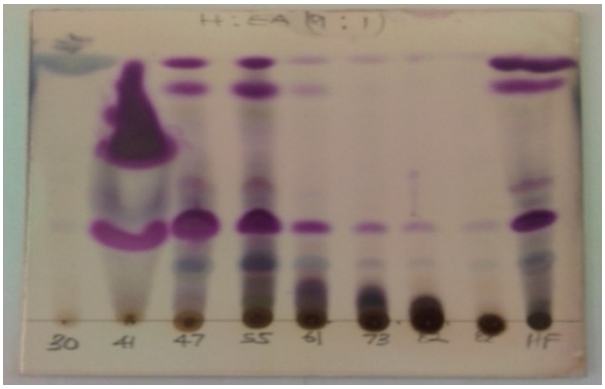

Plate IV: TLC of Pooled Column Fractions (PCFs) of HF and HF from Methanol Extract of *Vernonia cinerea* developed in Hexane: Ethyl acetate (9:1) and sprayed with *p*-anisaldehyde

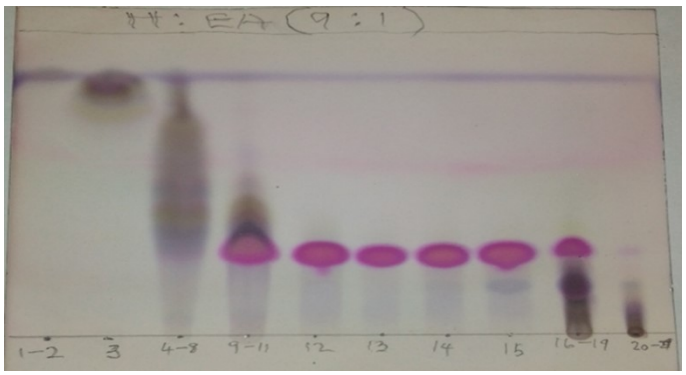

Plate V: TLC plate for Purification of HF developed in Hexane: Ethyl acetate (9:1) and sprayed with Leibermann Burchard

Table 2: Thin Layer Chromatography of Pooled Column Fractions of n-Hexane Fraction Compared with n-Hexane Fraction Developed in H: EA (9:1)

Fractions	Colour	No. of Spots
1 to 30	Ash	3
31 to 41	Deep purple, purple	3
42 to 47	Deep purple, light purple, blue	6
48 to 55	Deep purple, blue, ash	6
56 to 61	Purple, blue, brown	5
62 to 73	Purple, light blue, brown	5
74 to 82	Purple, blue, brown	4
83 to 88	Purple, light blue	2
HF	Deep purple, purple, blue, ash	6

Note: HF = n-hexane fraction, EA = ethyl acetate

Analysis of VC3

Physicochemical profile of VC3
The isolated compound; VC3, is a white flaky powder with a weight of 570 mg, it is soluble in chloroform and n-hexane solvent, it was also found to melt at a temperature range of between 222 to 286 ºC.

Thin layer chromatography (TLC) of VC3
VC3 was spotted on TLC plates, developed in n-Hexane: Ethyl acetate (9:1, 8:2 and 7;3), gave positive result with Leibermann Burchard (LB) reagent showing visible single spot (Plate VI) while negative results were recorded with Ferric chloride, Aluminium chloride, Bontragers and Dragendorff reagents.

Fournier Transform Infra-Red (FTIR) spectral data of VC3
The FTIR spectrum of VC3 showed peaks at 1039 cm^{-1}, 1699 cm^{-1}, 2866 cm^{-1}, 2933 cm^{-1}, 3075 cm^{-1} and 3362 cm^{-1} (Figure 3.1) and corresponding intensities, peak shapes and interpretations shown in Table 3.4

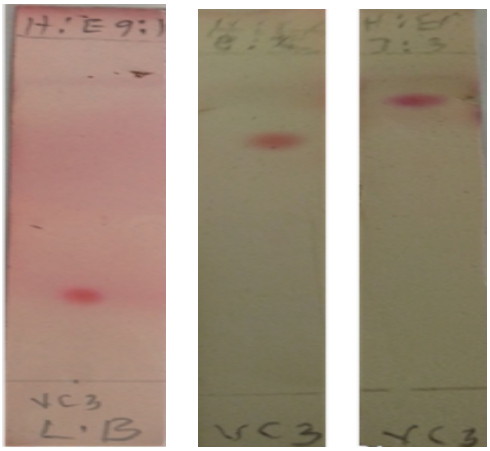

Plate VI: TLC of VC3 from n-Hexane Fraction developed in Hexane: Ethyl acetate (9:1, 8:2 and 7:3) and sprayed with Leibermann Burchard

Table 3.3: TLC Result of VC3 from HF of Methanol Extract of *Vernonia cinerea*

Solvent system	Colour	R_f Value
H: EA (9:1)	Pink	0.31
H: EA (8:2)	Pink	0.71
H: EA (7:3)	Pink	0.83

Note: HF= n-Hexane fraction, EA= Ethyl acetate

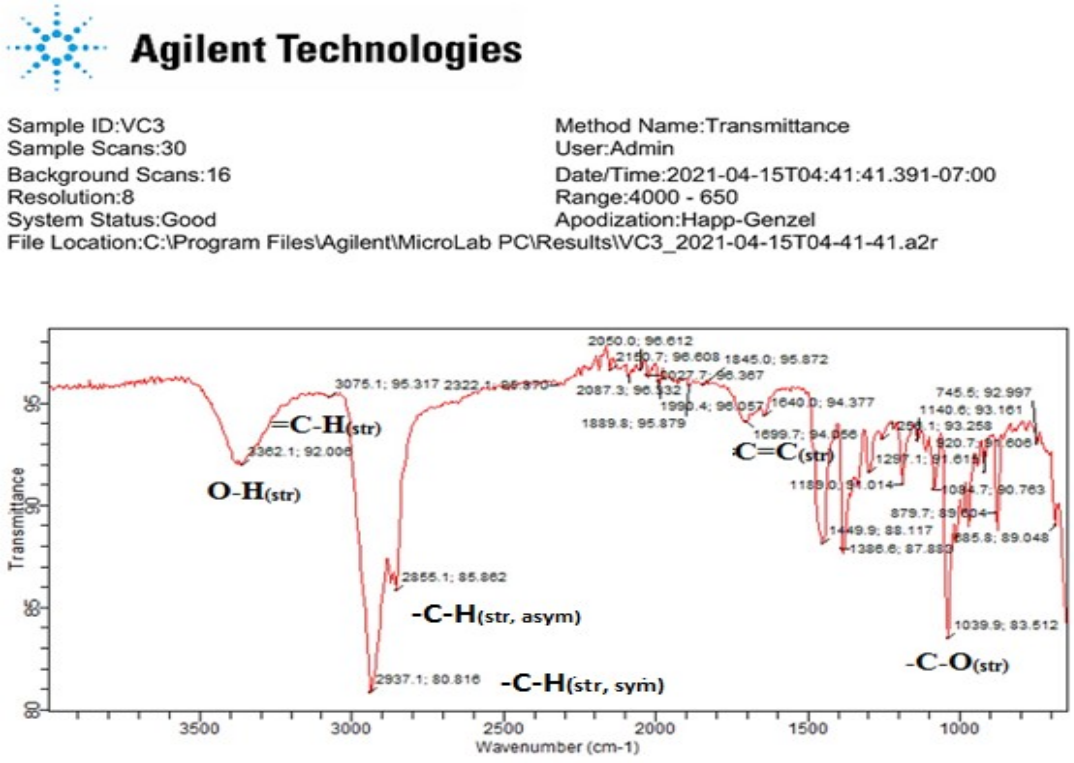

Figure 3.1: FTIR Spectrum of VC3 with Absorption Peaks at Various Regions

Table 3.4: FTIR Spectra of VC3

Wave numbers (cm-^{1})	**Functional groups**
3362	Free O-H
3075	=C-H stretch (alkenes)
2933	C-H stretch (Asymmetrical)
2866	C-H stretch (Symmetrical bending)
1699	C=C (cyclic stretch)
1039	C-O (stretch)

Nuclear Magnetic Resonance (NMR) Analysis of VC3

Figure 2 is the NMR signals from spectral results carried out on the isolated compound (VC3) for structural elucidation. Table 3.6 shows the results of hydrogen donation by HF, VC3 and ascorbic acid (AA) and acceptance by DPPH.

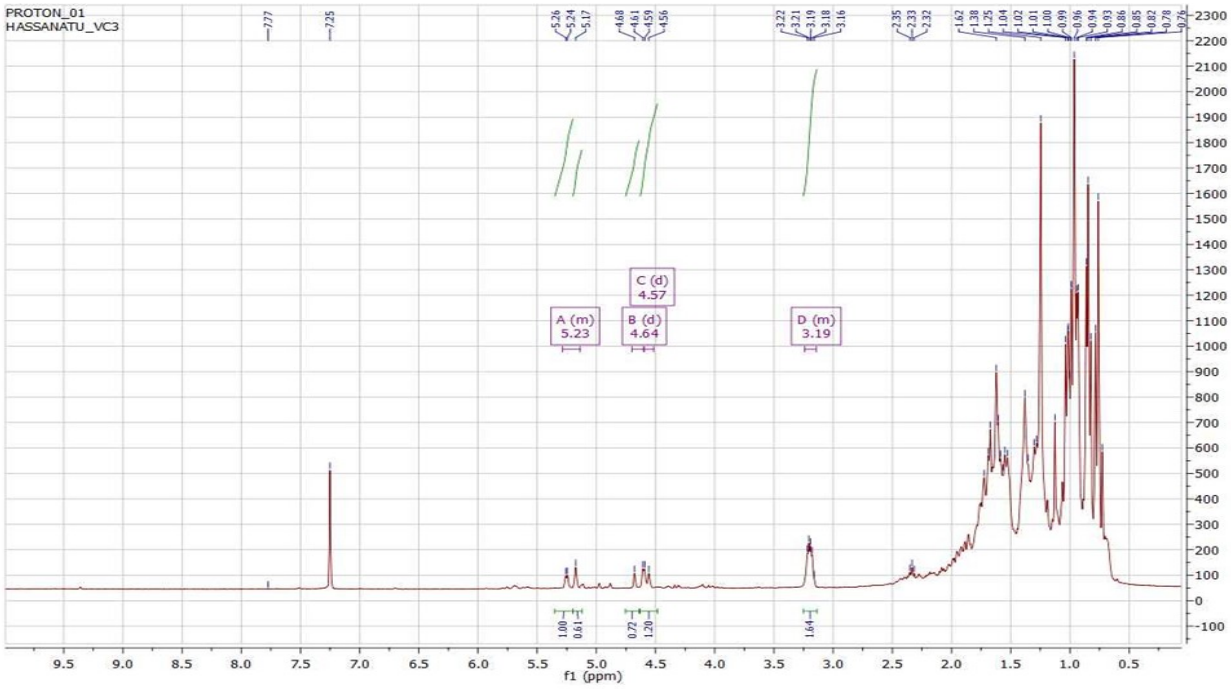

Figure 3.2: **^{1}H Spectrum of Compound VC3 in $CDCl_3$**

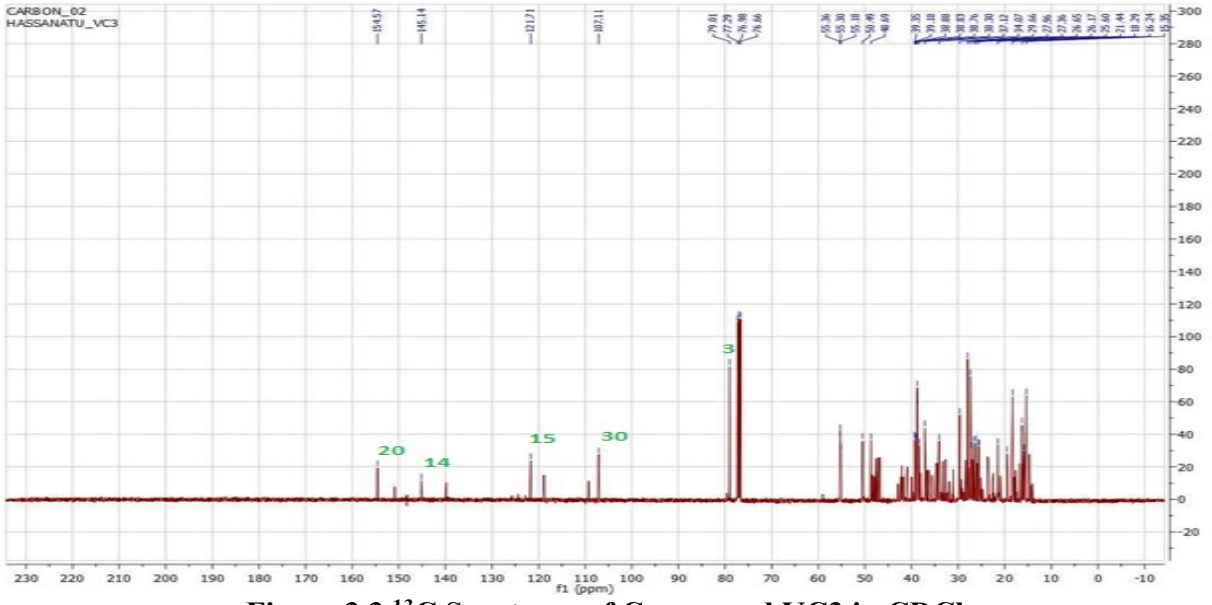

Figure 3.3 ^{13}C Spectrum of Compound VC3 in $CDCl_3$

Table 5: Comparison Between Chemical Shift Data of Taraxasterol and VC3 as obtained from Literature

Position	^{1}H Oladoye *et al*., 2015; Saritha and Prakash, 2018; Aduku *et al* 2020.	^{13}C	^{13}C VC3	^{1}H VC3
1	1.86	38.9	38.8	1.67
2	1.56(m)	27.5	27.3	1.57 (m)
3	3.21 dd(J= 11.4, 6.2 Hz)	79.1	79.0, **77.3***	3.19 (m, j= 4,8,12)
4		38.9	38.7	
5	0.79(m)	55.5	55.2	0.82
6	1.36(m, 2H)	18.4	18.3	1.35
7	1.37(m)	34.2	34.1	1.38
8		41.0	39.4	
9	1.33(s)	50.6	505	1.30
10		37.2	37.1	
11	1.52(m, 2H)	21.6	21.4	1.54
12	1.06(m)	26.3	26.2	1.04
13	1.59(m)	39.3	39.2	1.59
14		42.2, 145.4	39.4, **145.1***	
15	0.97(m), 5.31	26.8, 118,9	26.7, **121.7***	0.96, 5.23
16		38.4	38.4	
17		34.6	34.1	
18		48.8	48.7	
19	2.09(m, J=6.9 Hz, 1H)	39.5	39.2	2.32
20		154.7, 28.8	154.6, **29.7***	
21	2.19(m, J=15.9 Hz, 2H)	25.6	25.5	2.33
22	1.35(m)	39.0	38.7	1.30
23	1.01(s)	28.1	28.0	1.00
24	0.76(s)	15.4	15.4	0.76
25	0.92(s)	16.4	16.2	0.93
26	1.13(s)	16.0	15.9	1.13
27	0.97(s)	14.9	14.7	0.99
28	0.85(s)	19.6	19.5	0.85
29	1.20(d, J=4.0 Hz, 3H), 0.75	25.7, 33.3	25.6, **33.4***	1.26, 0.73
30	4.60brs, 4.62brs, 0.74	107.2, 21.3	107.1, **21.6***	4.64, 4.57, 0.73

Note: * Indicating corresponding data of Taraxarol at the same position with Taraxasterol

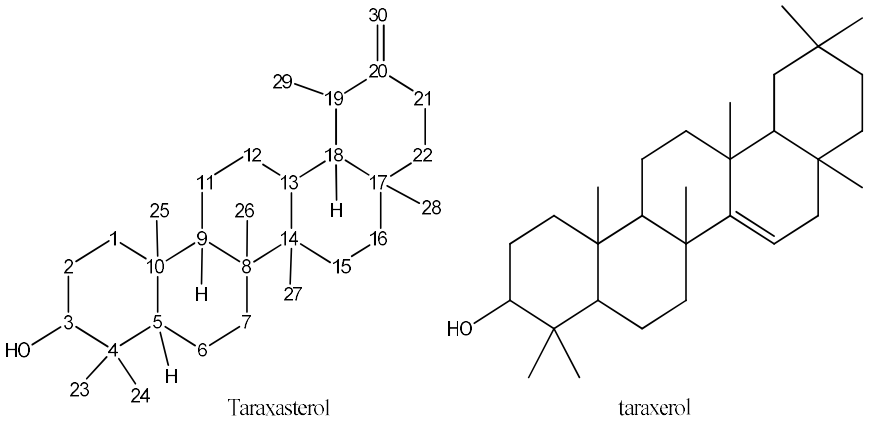

Figure 3.4: **Proposed Chemical Structures of VC3 (Mixtures of Taraxasterol and Taraxerol Evaluation of Antioxidant Activity of HF and VC3 from ME of Aerial Parts of *Vernonia cinerea***

Table 3.6: Hydrogen Donating Effect of HF, VC3 and AA on DPPH

		DPPH RSA (%)		
S/No	**Conc.**		**Mean ± SEM**	
	(µg / mL)	**HF**	**VC3**	**AA**
1	31.25	30.69 ± 0.25	29.58 ± 0.37	92.82 ± 0.40
2	62.50	35.00 ± 0.18	29.96 ± 0.19	95.61 ± 0.04
3	125	36.51 ± 0.26	30.11 ± 0.01	97.85 ± 0.03
4	250	38.58 ± 0.11	30.67 ± 0.15	98.98 ± 0.05
5	500	41.35 ± 0.12	33.43 ± 0.22	99.53 ± 0.03

Note: HF= n-hexane fraction, VC3= isolated compound, AA= ascorbic acid

Effect of n-Hexane Fraction from Methanol Extract of Aerial Parts of *Vernonia cinerea* and VC3 on β Hematin Formation

To determine the inhibitory effect of HF and VC3 on β hematin conversion to hemozoin, it was compared with an existing antimalarial drug (chloroquine), and the percentage β hematin inhibition of VC3 is slightly above that of HF and CQ (Table 3.7).

Table 3.7: β Hematin Formation by HF, VC3 and CQ

S/No	**Conc.**	**% Inhibition of β Hematin Formation (Mean ± SEM)**		
	(µg/mL)	**HF**	**VC3**	**CQ**
1	0.5	17.73 ± 0.57	13.68 ± 1.20	27.63 ± 1.08
2	1,0	28.73 ± 1.76	31.84 ± 0.72	43.28 ± 3.10
3	2.0	41.23 ± 0.65	51.37 ± 0.34	57.65 ± 2.52
4	4.0	49.01 ± 2.67	66.73 ± 2.03	62.37 ± 0.06

Note: HF= hexane fraction, VC3= isolated compound, CQ= chloroquine

DISCUSSION

In the *in vivo* antiplasmodial study of methanol extract from aerial parts of *Vernonia cinerea* (Isa *et al*., 2023), there was significant plasmodial clearance at the highest dose of the extract and HF. To know the active component that may be responsible for this effect, HF was purify to isolate VC3; a flaky white powder weighing 570 mg, which gave a single purple colour spot on TLC plate sprayed with *p*-anisaldehyde, a single pink colour spot to LB test for steroids / triterpenoids and a melting point range of 222-286 °C.

The results of the FTIR spectrum obtained for VC3 showed vibration at 3362 cm^{-1} which is an indication for free O-H stretching, vibration at 3075 cm^{-1} showed =C-H stretching of alkene, 2933 cm^{-1} is a vibration of C-H symmetrical stretching, then, vibration at 2866 cm^{-1} indicated C-H of asymmetrical bending, 1699 cm^{-1} is a region for aromatic stretching vibration of -C=C- and 1039 cm^{-1} is a stretching vibration due to the presence of –C-O.

However, a literature search found that taraxasterol (3β, 18α, 19α)-Urs-20 (30)-en-3- ol) and taraxerol (3b) −D‑Friedoolean‑14‑en‑3‑ol) are both pentacylic triterpenoids with same molecular formula and molecular weight with variation in their melting points (MPs), taraxasterol presented a sharp MP of 221–222°C (Jiao *et al*., 2022) while taraxerol have MP range of between 282-285ºC (Sharma and Zafar, 2015). From above, the MP of VC3 is beyond the range of ± 3 for pure compound, which indicates that VC3 might be a mixture with MP of 222-286 °C is within the range of MPs of taraxasterol and taraxerol.

The ^{1}H NMR spectrum revealed the presence of several overlapping signals upfield due to methyl and methylene protons, these are features well-known with steroid and triterpenoid nucleus (Shehu *et al.,* 2015). It also revealed a signal δ_H3.19 ppm (1H, dd, J = 12, 4.8 Hz) due to carbinol proton at the H-3 proton at position C-3. This is also a deshielded proton as a result of the OH group, a typical characteristic of steroids and triterpenoids (Shehu *et al.,* 2015). The spectrum also displays a set of broad singlets at δ 4.57 and 4.64, with additional 5.23 characteristic of olefinic protons (H-30a and b and H-15) with an additional 0.73 s and 0.74 s typical of terminal methyl groups indication that the compound may be a mixture (Saritha and Prakash, 2018). The spectrum also showed 7 signals typical of tertiary methyl groups of which 6 were singlets (δ 0.73, 0.85, 0.93 0.99, 1.13, and 1.25) and one doublet at 1.26, characteristic of triterpenoids and are in good agreement with that of taraxasterol (4,4,6a,6b,8a,12,14b-heptamethyl-11-methylidene-docosahydropicen-3-ol) (Alavi and Yekta, 2008; Haba *et al.,* 2012).

The ^{13}C NMR spectrum revealed a total of 30 prominent carbon resonances including a deshielded secondary hydroxyl bearing carbon at δ_C 79.16 with an additional 77.3, quaternary carbon signals including a highly deshielded carbon signal at δ_C 154.5 (C-20), 145.1 (C-14) and 7 methyl groups (Alavi and Yekta, 2008, Saritha and Prakash, 2018). Taraxerol differs from taraxasterol in the following positions; oleifinic group between carbons 14 and 15, and qurtenary carbon (20) (Oladoye *et al*., 2015).

These triterpenoids were previously isolated from many plants of Asteraceae family; *Centaurea omphalotricha*, *Oxystelma esculentum***,** *Centaurea kilaea*, *Centaurea perrottetii*, *Taraxacum officinale*, *Calendula officinalis*, *Carthamus lanatus*, *Hieracium pilosella*, *Mikania cordifolia*, *Achillea millefolium*, *Cichorium glandulosum*, *Centipeda minima*, *Chrysanthemum morifolium*, *Arctium lappa* etc. (Haba *et al*.,

2012; Sharma and Zafar, 2015; Sen *et al*., 2017; Aduku *et al*., 2020; Jiao *et al*., 2022). Other plant families from which these phytoconstituents were also isolated include: *Camellia japonica* (Theaceae), *Acrocarpus fraxinifolius* (Fabaceae), *Holodiscus discolor* (Rosaceae), *Strobilanthes callosus* (Acanthaceae), *Philadelphus coronarius* (Hydrangeaceae), *Bryophyllum pinnatum* (Crassulaceae), *Cornus kousa* (Cornaceae), *Solanum lycopersicum* (Solanaceae), *Euphorbia tirucalli* (Euphorbiaceae), *Olea europaea* (Oleaceae), *Ficus carica* (Moraceae) etc. (Jiao *et al*., 2022). These compounds are treasured for their pharmacological activities such as; anti-inflammatory, anti-oxidative, anti-protozoal, anti- carcinogenic, diuretics, hypoglycemia, antimicrobial activities, anti-snake venom etc (Sharma and Zafar, 2015; Prashant *et al*., 2021; Jiao *et al*., 2022).

In the antioxidant studies of HF and VC3 from ME of *V. cinerea*, the scavenging activity was maximum at 500 µg / mL concentration for HF (41.35 %) and VC3 (33.57%) when compared to AA which gave 99.53 % scavenging activity. The DPPH scavenging effect in the HF is slightly higher and dose-dependent than VC3 (Table 4.30), is an indication that it may contain more than one dissolved substance with antioxidant tendency. This result is not considered significant, because HF and VC3 showed low proton donating ability when compared to AA, which may be due to the nature of fraction and isolated compound. Besides, polar compounds such as polyphenols (Keyata *et al*., 2021) are known to have higher antioxidant effect than non-polar or slightly polar fractions and compounds. With the results obtained, HF and VC3 may possibly act as primary antioxidant. The use of an antioxidant (zinc) as a supplement in malaria infection is known to confer protection to the host through membrane stabilization of erythrocytes and inhibition of parasite progression of destroying uninfected red blood cells and not the parasite membrane (Irihboghe *et al*., 2014). To enhance the antioxidant and immune response of the host against malaria, recent studies showed significant antioxidant compounds from sources such as plants among others, which act by modulating the host's response, strengthening the endogenous antioxidant defense against oxidative stress or acting indirectly towards parasite destruction (Gomes *et al*., 2022). A study demonstrated the combination of artemisinin and flavonoids from *Artemisia annua* and some other promising plants tested, showed high antimalarial and/or antioxidant activity (Camara *et al*., 2019). This is a pointer that an increase in the body's antioxidants from external sources can initiate a decrease in parasite number and alleviate severe infection in the long run (Marjoni and Zulfisa *et al*., 2017).

As for the inhibition of conversion of heme to hemozoin, both HF and VC3 inhibited β hematin formation in a dose dependent manner in comparison with the positive control. Highest percentage inhibition was seen in the 4 µg/mL concentration for CQ, HF and VC3 (62.37, 49.01 and 66.55 %) respectively. VC3 is the isolated compound, and it showed β hematin inhibition higher than the CQ and HF at the same concentration which is suggestive that VC3 may be acting through the same pathway as the positive standard with probable antiplasmodial activity. The model of β-hematin inhibition is considered an excellent tool for screening of herbs with antimalarial potential in *in vitro* test (Abiodun, 2018). Previous studies showed quinine and artemisinin as drugs from natural sources with β hematin inhibition effect (Babatunde and Wande, 2017). The use of phytochemicals in medicine is well known, because most of the currently used

pharmaceuticals are derived from natural substances, they serve as resources for creating novel medicines for management of diseases (Noureddine and Lahcen, 2024). There is need to discover a single compound with more than one biological effect to reduce cost and allow for a simpler treatment plan (David *et al*., 2015). The isolated compound may be explored for the development of new phytotherapy for the treatment of malarial infection.

CONCLUSION

Both the n-hexane fraction and VC3 were from aerial parts methanol extract of *Vernonia cinerea*, they showed low antioxidant effect due to non-polar nature of the fraction and its isolated compound. However, inhibition of β hematin formation by VC3 is higher than chloroquine (CQ); the standard drug and n-hexane fraction, this indicate that VC3 may be acting through the same pathway as other previously used antimalarial drug.

Conflict of Interest Statement

The authors declare no conflict of interest.

Acknowledgements

The authors are thankful to Mr Silas Ekwuribe of Multi-user Science Research Laboratory, Department of Chemistry and Dr. Mansir Aliyu of the Department of Biochemistry, all of ABU Zaria for their technical assistance.

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