International Research Journal of Pure and Applied Chemistry

Volume 25, Issue 3, Page 76-89, 2024; Article no.IRJPAC.115202
ISSN: 2231-3443, NLM ID: 101647669

Isolation and Characterization of Glucocorticoid Steriod from the Leaf of *Rauvolfia vomitoria*

Uyanwa Maryjane N. [a*], Okenwa Uchenna [b], Iwu Irenus [a], Nwoke Linda [c] and Echeme Johnbull O. [b]

[a] Department of Chemistry, Federal University of Technology, Owerri, Nigeria.
[b] Department of Chemistry, Michael Okpara University of Agriculture Umudike, Nigeria.
[c] Department of Chemistry, Federal College of Agriculture Isiagu, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2024/v25i3858

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115202

Original Research Article

Received: 07/02/2024
Accepted: 11/04/2024
Published: 06/05/2024

ABSTRACT

Isolation and characterization of glucocorticoid steroid was carried out on the leaf of *Rauvolfia vomitoria*. Initial phytochemical screening of the sample revealed the presence of saponins, flavonoids, alkaloids, tannins, steroids, triterpenoids and cardiac glycoside and were quantitatively determined as follows; saponins (2.37 ± 1.09), tannins (0.09 ± 0.01), alkaloids (0.82 ± 1.05), and flavonoids (0.82 ± 0.05). Isolation of the compound was carried out with the aid of column chromatography and isolate subjected to thin layer chromatography. Sample was subjected to spectroscopic analysis with the aid of FT-IR, ^{1}H NMR, ^{13}C NMR and HSQC. The structure of compound was determined as Cis-3-(2,2-dimethyl-26-(((2S,3R,4S,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl)-8-methoxy-15-(S)-3-methoxy-4,4-dimethylpentyl)-14-(3-methoxy-4,4-dimethylpent-1-yn-1-yl)-1,2,3,4,11,12,13,14,15,15a-octahydrophenanthro [2,1-b]furan-16(10H)-one with a molecular formulae of $C_{44}H_{68}O_{11}$. A glucocorticoid steroid.

**Corresponding author: E-mail: nkiscomju@yahoo.com;*

Keywords: Steroids; phytochemicals; column chromatography; glucocorticoids; spectroscopy.

1. INTRODUCTION

The World Health Organization recognizes medicinal plant parts such as; leaves, roots, rhizomes, stems, barks, flowers, fruits, grains, or seeds as having therapeutic values. These parts are used in the management or treatment of disease conditions. The most useful chemical components are phytochemicals which are secondary plant metabolites [1,2,3,4]. Medicinal plants has been used to treat a variety of conditions, such as fever, asthma, constipation, esophageal cancer, and hypertension [5]. *Rauvolfia vomitoria* Afzel is mostly found in regenerating forests where extended periods of fallow are prevalent. It is a member of the Apocynaceae family. Within the Apocynaceae family, *R. vomitoria* Afzel is a small tree or big shrub that can reach a height of 15 meters. It features shiny, oval, or oblong leaves arranged in whorls, straight veins, and a cluster of small, white, or greenish flowers that yield red berries. While the Igbo people in Nigeria and the Ashantes people in Ghana called *R. vomitoria,* wada, its name is mmoneba (Efik), and utoenyin (Ibibio), respectively. The Yoruba people in Nigeria refer to it as "Asofeyeje," which means bearing fruits for the birds [6,7,8]. The plant belongs to two distinct species: *R. vomitoria,* which is African variety, and *R. serpentina,* which is Indian variety [9]. In natural medicine, this plant is also known for its several medicinal benefits, which include alleviating male infertility, hypertension, malaria, and mental disorders, among other conditions [10]. The leaf of the plant is said to contain alkaloids, saponins, and flavonoids [11]. Though this plant has so much medicinal applications, its constituents are not fully characterized.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

The leaves of *R. vomitoria* were obtained in December 2021 from a thicket near Yaradua Drive in Owerri, Imo state, in eastern Nigeria. The plant sample, bearing authentication number UBHR421, was identified by Mr. Obiajunwa, Udoka of Forest Ecology and Conservation in the Department of Forestry and Environmental Management at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were air-dried, coarsely ground with a hand blender, and then stored in an airtight container. 800 g of the sample was measured into a glass jars and 3.8 litres of analytical-grade chloroform solvent were added until the leaf samples were fully submerged. After that, the glass jars were securely sealed and stored for five days in a secure location. Using Whatman No. 1 filter paper, the extract was filtered out of the solution after the extraction procedure. The solvent and extract mixture were separated using a rotary evaporator.

2.2 Partitioning of the Crude Chloroform Extract

20 g of the crude chloroform extract of the sample was partitioned using 200 cm^3 of chloroform and 200 cm^3 of distilled water. The crude extract was dissolved first with the solvent which was continuously stirred to ensure complete dissolution and 200 cm^3 of water was added. The mixture was then poured into a separation funnel, shaken vigorously until a homogenous mixture was obtained and left to stand for 24 hours to separate. The chloroform extract was allowed to evaporate completely. The dried chloroform fraction was mixed with 50 g silica gel (200 mesh) to form a homogenous mixture called slurry for column chromatography.

2.3 Column Chromatography

The glass column was washed and rinsed with petroleum ether. A plug of cotton wool was introduced into the glass column and was pushed to the neck of the column with a steel rod to prevent silica gel from entering into the collection vials and to provide an even baseline for the slurry. The silica gel (200 mesh) was mixed with petroleum ether and poured into the column. This was repeated until a lengthy column was obtained. The column was allowed to drop to ascertain the smooth flow of the solvent. Then the slurry was gently introduced into the column and 100 cm^3 of petroleum ether was added gradually to wash down the sides of the column. After which silica gel was introduced to prevent disturbance to the plant sample when pouring the different solvents. The collection of fractions in 40 cm^3 was carried out with 100 cm^3 beakers after the introduction of 100 cm^3 petroleum ether followed by introduction of solvent mixture of petroleum ether and chloroform (90:10). The column was gently tapped with a rubber hose to release trapped

air bubbles in order to forestall column cracking. After which the process continued with petroleum ether: chloroform (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90) and 100 cm^3 chloroform. Then chloroform and ethyl acetate (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90) and 100 cm^3 ethyl acetate. Followed by ethyl acetate and methanol (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90) and lastly 100 cm^3 methanol. 68 fractions were collected in total.

2.4 Thin Layer Chromatography

The sample fractions were subjected to preliminary Thin Layer chromatography (TLC) which was used to determine the purity and number of compounds that could be present in each of the fractions collected from column chromatography. With the use of a capillary tube, each of the fractions was applied on pre-coated aluminum thin-layer chromatographic plates and at half inch apart from the lower edge of the plate. The plates were kept in different developing chambers of 250 cm^3 beakers containing solvent mixtures for specific time until the developing solvent reaches top of the upper edge of the TLC plate. The plates were taken out from developing chamber, dried and solvent front was marked with lead pencil. The compound bands/spots on TLC chromatographic plates were visualized in Iodine chamber and marked. The retention factor (R_f) of each spot was calculated using the formula;

$$\mathrm{Rf} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

Afterward, the sample fraction was obtained with R_f value of 0.75. The fraction was subjected to spectral analysis such as Infra-red (IR) and Nuclear Magnetic Resonance (NMR).

2.5 Screening for Alkaloid

5 g of the sample was extracted using 20 % acetic acid in ethanol. 5 cm3 of the extract was treated with Wagner's reagent (iodine crystals and KI). A yellowish brown precipitate indicates the presence of alkaloid. Mayer's reagent (potassium mercuric iodide) was added to 10cm^3 of the filtrate. Alkaloids were present when a yellow-colored precipitate formed [12].

2.6 Screening for Saponins

Froth Test: 10 g of sample was dissolved in 100 cm^3 of water and extracted after 4 hours. 10 cm^3 of the extract was measured into a 50 cm^3 beaker and shaken vigorously. The formation of a 1 cm layer of foam suggested the presence of saponins [12].

2.7 Screening for Cardiac Glycosides

Chloroform was used to treat the extract before it was filtered. The filtrate was then heated and cooled shortly after being treated with a few drops of acetic anhydride. Sulfuric acid concentrate was added. The presence of cardiac glycosides was revealed by the formation of a brown ring at the junction [12, 13].

2.8 Screening for Steroids

Salkowski's Test: Chloroform treatment and filtering were applied to the extract. To create a lower layer, a few drops of concentrated sulfuric acid were then added to the filtrate. The presence of steroids were indicated by a golden-yellow appearance.

2.9 Screening for Tannins

Gelatin Test: A 1% sodium chloride-containing gelatin solution was added to the water extract. The presence of tannins was revealed by the formation of a white precipitate [13].

2.10 Screening for Flavonoids

Shinoda's Test: The extract was dissolved in ethanol, and the filtrate was then mixed with a few drops of conc. HCl. After that, magnesium tunings were added. Flavonoids were detected by the formation of a pink-red coloring [12].

2.11 Screening for Terpenes

After adding 3 cm^3 of chloroform to the extract, it was filtered. 3 cm^3 of concentrated sulfuric acid was added to the filtrate. Pink colouraton of the interphase indicates the presence of terpenes [12].

2.12 Alkaloids Determination

A 250 cm^3 beaker was filled with 15 g of the sample, 200 cm^3 of 20% acetic acid in ethanol, and agitated. The beaker was then capped and allowed to stand for four hours. The Whatman filter paper was used to filter the mixture, and a water bath was used to concentrate the extract to a quarter of its initial volume. Concentrated ammonium hydroxide was applied drop by drop

until the precipitation process was completed to remove the alkaloid. Using weighed Whatman filter paper, the precipitate was collected, using the filtering process. After dried and the precipitate was weighed again.

2.13 Saponins Determination

200 cm^3 of a 20 % aqueous ethanol solution was added to a 250 cm^3 beaker containing 15 g of the sample, which was then swirled with a glass rod. The mixture was heated over a water bath for four hours, stirring continuously to keep the temperature at 55 °C. The Whatman filter paper was used to filter the mixture, and 200 cm^3 of 20 % aqueous ethanol solution was used to extract the residue for one hour. The extracts were combined and boiled in a water bath at 90 ^0C until they weighed about 40 cm^3. After transferring the concentrated extract into a 250 cm^3 separation funnel, 20 cm^3 of diethyl ether was added, and the mixture was vigorously shaken. The ether layer was laid out, and partitions were formed and so that the aqueous layer could then be recovered. Three times this technique was carried out, and n-butanol (60 cm^3) was added. 10 cm^3 of 5 % sodium chloride were used to wash the mixture twice. In a weighed evaporation dish, the residual solution was heated to dryness over a water bath and then reweighed. The saponin content was determined.

2.14 Flavonoids Determination

15 g of the plant material was extracted several times using 100 cm^3 of 80 % aqueous methanol. Whatman filter paper No. 45 was used to filter the resultant solution. After being poured into a crucible, the filtrate was dried over a water bath and weighed [14]. The flavonoid content was calculated.

2.15 Determination of Tannins

A beaker was filled with 15 g of the sample. It was mixed with 150 cm3 of distilled water and allowed for four hours. After placing a stirring rod and allowing the liquid to sit for four hours, the Whatman filter paper was used to filter. The mixture was filtered and a few drops of concentrated hydrochloric acid was added to the filtrate to make it more acidic, then ethyl acetate was added. Using a separating funnel, this solution was thoroughly shaken and partitioned. The ethyl acetate layer was disposed of and an aqueous solution was produced. Following three repetitions of this, the aqueous solution was boiled to dryness, and the tannins that were produced were weighed. The quantity of tannins were calculated.

3. RESULTS AND DISCUSSION

3.1 Qualitative Screening of *R. vomitoria* leaf

The result of the initial assessment of the phytochemical composition of *R. vomitoria* leaves is contained in Table 1 below.

Table 1. Result of phytochemical screening of *R. vomitoria* leaves

Phytochemical	Inference
Saponins	++
Flavonoids	++
Tannins	++
Alkaloids	++
Cardiac glycosides	+
Steroids	++
Terpenoids	++

Key; ++ present, + slightly present, – absent

The results of the qualitative phytochemical screening of the R. vomitoria leaf extract reveals the presence of saponins, flavonoids, alkaloids, tannins, steroids, and triterpenoids, with a slight presence of cardiac glycoside (11). Most of these compounds were quantitatively determined in Table 2.

Table 2 shows that the extract from *R. vomitoria* leaves includes different levels of tannins, alkaloids, flavonoids, and saponins. When compared to alkaloids and flavonoids, tannins have the lowest concentration in *R. vomitoria* leaf extract, whereas saponins have a substantially larger concentration. The majority of phytochemicals are present in plant-based diets; they are absent from dairy and animal products. Most green leafy plants have been reported to contain alkaloids, which have a wide range of medicinal uses. These are secondary metabolites that have long since been discovered to be crucial for human health. They are found in foods and beverages consumed by humans daily, as well as in stimulant medications. According to [15], the majority of alkaloids have been used medicinally for their antimalarial, antioxidative, antibacterial, anti-inflammatory, anticancer, antiasthma, vasodilatory, analgesic, and neuropharmacological properties. Because of the alkaloid concentration in the leaf extract, *R. vomitoria* leaves are used to treat fever, hypertension, jaundice, and rheumatism.

Table 2. Result of the quantification of *R. vomitoria* leaves

Phytochemical	Concentration mg/100g
Alkaloids	0.82 ± 1.05
Saponins	2.37 ± 1.09
Flavonoids	0.82 ± 0.05
Tannins	0.09 ± 0.01

Values are means ± standard deviation of triplicate determination

Groups of secondary metabolites of polyphenolic plants are called flavonoids. They add to the color of herbs, vegetables, and fruits. Among the bioactive flavonoids are quercetin, which has antibacterial, antiviral, anticarcinogenic, and anti-inflammatory properties, [16], and hesperidin, which has an antihypertensive and lipid-lowering impact [17]. Therefore, these properties justify the application of *R. vomitoria* leaves in inflammatory and cancer therapies [18].

Among the phytochemicals found in plants are saponins. These are chemical substances found in many different kinds of plants, seeds, and herbs. It was discovered that *R. vomitoria* leaves have a high availability of saponins. They boast several health benefits and can foam when combined with water. To control immunological responses, saponins are used in vaccination formulations. By protecting the body from bacteria, viruses, and fungi, saponins have antibacterial, antiinflammatory, and immune-boosting qualities. They scavenge oxidative stress and function as antioxidants. Their hypoglycemic properties help to minimize insulin surges and maintain normal blood sugar levels. Saponins can reduce body fat and cholesterol, which helps to improve cardiovascular health. Both humans and animals have shown them to have a cholesterol-lowering effect [19]. Many biological and therapeutic effects, including hemolysis, molluscicidal, anthelmintic, insecticidal, analgesic, antiviral, anticancer, sedative, and anti-tumor activity, have been found for saponins [20]. It is widely believed that they are crucial to plants' defense against illnesses and insects. They are also utilized in the food supplement and cosmetic (soap, emulsions) industries, as well as in several pharmaceutical and medicinal applications [21]. *R. vomitoria* leaves contain saponin, which makes them useful for treating conditions including headaches, inflammation, high blood pressure, and chest discomfort.

Plants such as *R. vomitoria* include flavonoids, alkaloids, and saponins that have enzymatic, antioxidant, anti-inflammatory, and immunomodulatory properties that can either delay or improve rheumatoid arthritis.

One type of phytochemical in the plant extract from *R. vomitoria* is steroids. They support numerous therapeutic uses, including those of cardiotonic (digitoxin), vitamin D precursors (ergosterol), oral contraceptives (semisynthetic estrogens and progestins), anabolics (androgens), and anti-inflammatory (corticosteroids). Since inflammation is the primary cause of asthma, corticosteroids are mostly used to treat severe cases of the condition [22]. This suggests that *R. vomitoria* plant leaves may be used similarly to treat inflammatory illnesses by acting as an antiinflammatory drug.

Tannins are a type of polyphenol that has several therapeutic and medical uses. They also function as antioxidants and have some pharmacological qualities, including the ability to heal wounds, prevent dysentery, fight bacteria, reduce inflammation, and be anti-toxic, anti-cancerous, antiallergic, and anti-inflammatory [23]. In addition, tannins have several negative consequences, such as increased indigestibility, antinutritional effects, mutagenesis, and carcinogenic properties, ability to cause and co-promote several diseases, migraine intensity, hepatotoxic activity, and inhibitory actions when consumed in large quantities. Additionally, they have been used to treat throat infections, hemorrhoids, diarrhea, and dysentery [24]. Since tannin was discovered to be somewhat prominent in *R. vomitoria* leaves, the plant's leaves may therefore be used as a treatment for the aforementioned illnesses.

Cardiac glycosides are steroid-based medications that lower heart rate and increase heart muscle contraction force without increasing oxygen consumption in the treatment of congestive heart failure and cardiac arrhythmia. Apart from their beneficial effects on heart health, cardiac glycosides also exhibit anticancer capabilities [25]. They do this by activating the heart muscle, which enhances cardiac output and efficiency. They also aid in the deceleration of irregular heartbeats. They are found in trace levels in the leaves, seeds, stems, roots, and bark of widely distributed plants. It was discovered that *R. vomitoria* leaves had a small amount of cardiac glycoside, which may be used to treat cardiovascular illnesses.

Triterpenes are found in several ethnomedicinal plants, including the leaves of *R. vomitoria*. Traditional healers regularly recommend herbal medicines containing triterpenes to treat or prevent a wide range of illnesses in several Asian nations [26,27]. In nutraceutical products, triterpenes can reduce pain and have anti-inflammatory, antiviral, antibacterial, anticancer, and immunomodulatory properties. As a result, *R. vomitoria* leaves can be used to treat a variety of illnesses, including malaria, rheumatism, diarrhea, and fever.

3.2 Characterization of Compound

The Compound was proposed from the complete elucidation of spectroscopic parameters which include; FT-IR, ^{1}H NMR, ^{13}C NMR, and HSQC.

3.3 FT-IR Spectral Data of Compound

The spectrum of compound is presented in Fig. 1.

The interpretation of the FT-IR spectra of compound is shown in Table 3.
Table 3 shows data from the FT-IR spectrum of compound. The strong, broad, and intense absorption band at 3378.13 cm-1 depicted an O-H stretching of an alcohol. The broadness of the band is due to the intermolecular hydrogen bond that exists among the O-H groups of the sugar moiety [28]. The sharp and intense absorption bands at 2921.71 cm^{-1} and 2850.93 cm^{-1} depicted the presence of C-H stretching vibration of an aliphatic group, which implies that hydrogen was attached to sp^3 carbons. A medium and sharp absorption band at 2251.40 cm^{-1} indicated the presence of a substituted C≡C stretching vibration of an alkyne group. The strong and intense absorption at 1719.98 cm^{-1} indicated the presence of a C=O stretching vibration of an ester [29]. Bands at 1648.60 cm^{-1},1625.30 cm^{-1} and 1550.00 cm^{-1} with medium and sharp intensity, depicted the presence of a C=C stretching of aromatic [30]. Also, the medium and sharp bands at 1463.73 cm^{-1} and 1377.83 cm^{-1}, depicted the C-H deformation, that is, bending vibration of -CH_2 and -CH_3 respectively. Again, the medium and broad bands at 1162.89 cm^{-1}, 1072.98 cm,$^{-1,}$ and 1063.00 cm^{-1} indicated the presence of a C-O stretching of an alcohol and an ether. The medium and sharp bands at 912.94 cm^{-1}, 741.15 cm,$^{-1,}$ and 647.78 cm^{-1}, also, depicted a C-H bending vibration of aromatic [31]. presented in Fig. 3.

Fig. 2: ^{1}H NMR spectrum of compound

3.4 ^{1}H NMR and ^{13}C NMR of Compound

The ^{1}H NMR spectrum of compound is shown in Fig. 2 while the ^{13}C NMR spectrum is

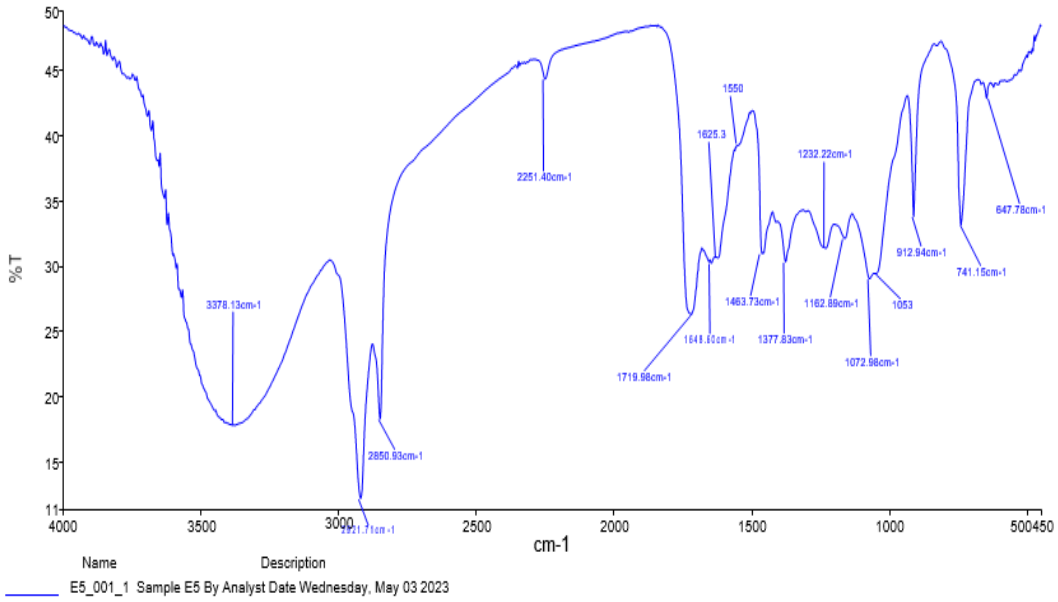

Fig. 1. FTIR Spectrum of compound

Table 3. Interpretation of FT-IR spectra for compound

Frequency of Absorption (cm^{-})	Characteristics/Intensity	Type of bond/Vibration
3378.13	broad and intense	-O-H stretching vibration of an alcohol
2921.71 2850.93	sharp and intense	-C-H stretching vibration of an aliphatic
2251.40	medium and sharp	C≡C stretching vibration of an alkyne
1719.98	medium and sharp	C=O stretching of an ester
1648.60 1625.30 1550.00	medium and sharp	C=C stretching of an aromatic
1463.73 1377.83	medium and sharp	C-H deformation of CH_2 and CH_3
1162.89 1072.98 1053.00	medium and broad	C-O stretching vibration of an alcohol and an ether
912.94 741.15 647.78	medium and sharp	C-H deformation of an aromatic

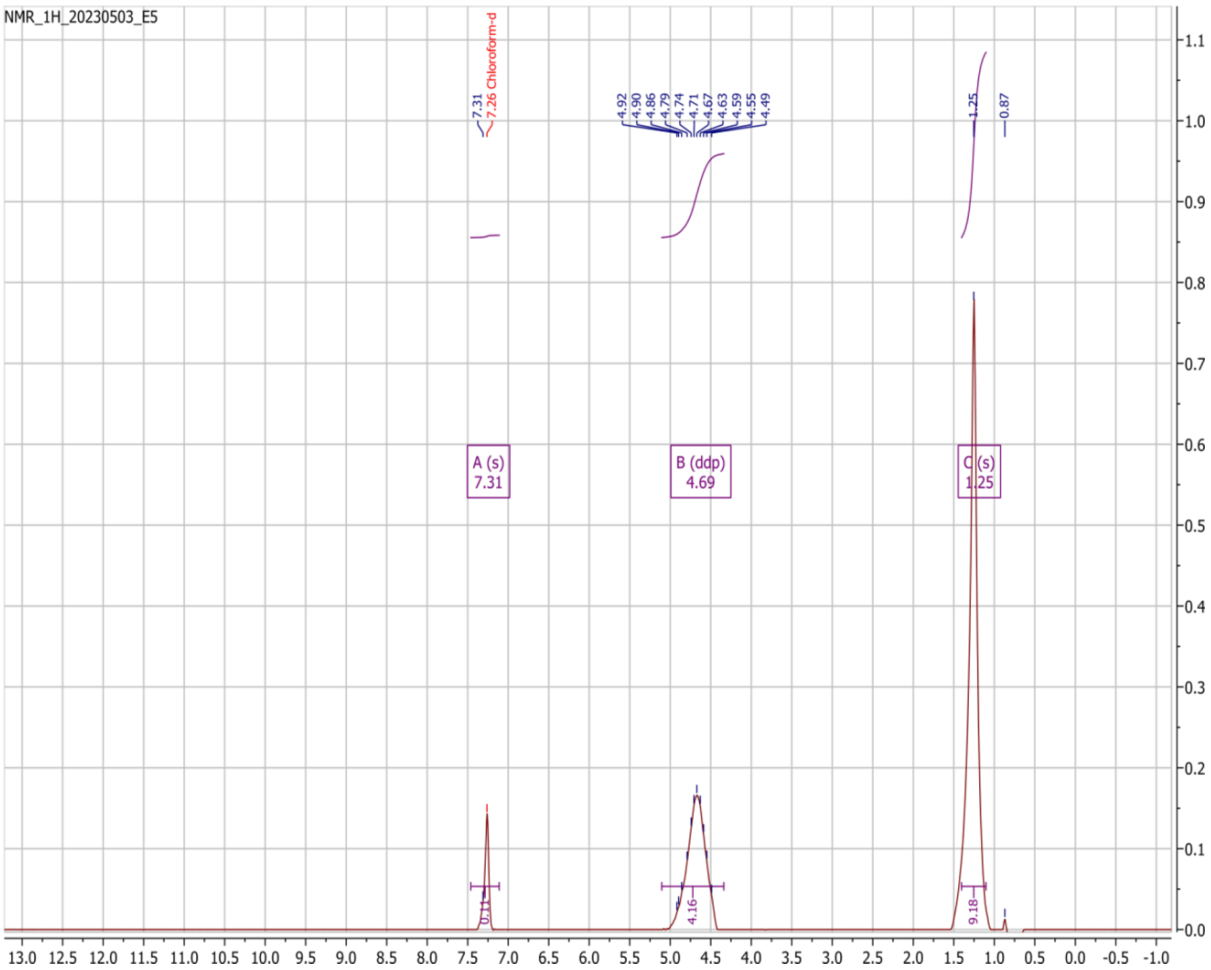

Fig. 2. ^{1}H NMR spectrum of compound

Fig. 3. ^{13}C NMR spectrum of compound

The interpretation of the ^{1}H NMR and ^{13}C NMR spectra of compound are shown in Table 4.

^{1}H NMR spectrum of compound revealed different kinds of proton present in compound E5. It also showed a cluster of peaks between 0.50 ppm to 2.50 ppm, which is an indication that compound is a steroid, and a cluster between 3.50 ppm to 6.00 ppm, which is also an indication that compound contains a sugar moiety [28]. The protons revealed by the spectrum include; methyl proton (CH_3) presented in positions H-22, H-23, H-24, H-28, H-29, H-30, H-36, H-37, and H-38 with a resonance frequency signal at δ 1.25 ppm. The protons are chemically and magnetically equivalent which appeared as singlet (s), shielded by electron density, and also in the same environment. Methoxy protons (O-CH_3) in positions H-21 and H-35 are in the same environment and thereby resonated at δ 3.51 ppm, experienced de-shielding also, by inductive effect. H-25 (OCH_3) at δ 3.71 ppm is also de-shielded by inductive effect [32-34].

Another type of proton revealed by the spectrum is methylene proton (CH_2) presented in positions H-1 at δ 1.60 ppm with a triplet (t) multiplicity. It is in an electron-rich environment. H-2 which resonated at δ 1.42 ppm with a quartet (t) multiplicity, is also a methylene proton that is shielded by electron density. H-4 at δ 1.52 ppm, with a doublet (d), is shielded by electrons. H-13 at δ 1.63 ppm with the integration of 2H and splitting pattern of triplet (t), is also, shielded by electrons. H- 17 and H-18 at δ 1.95 ppm and δ 2.00 ppm with a quartet (q) and a triplet (t) respectively, are also methylene protons in an electron-dense environment [35-37].

Table 4. ^{1}H NMR and ^{13}C NMR of compound

Position	δC Chemical Shift Value(ppm)	Type of Carbon	δH Chemical Shift Value(ppm)	NO. of Proton	Multiplicity
1	30.63	CH_2	1.60	2H	T
2	23.18	CH_2	1.42	2H	Q
3	34.15	CH	1.71	1H	S
4	36.87	CH_2	1.52	2H	D
5	120.78	Ar-C	-	-	-
6	120.78	Ar-C	-	-	-
7	109.55	Ar-H	7.31	1H	S
8	135.03	AR-C-O	-	-	-
9	120.78	Ar-C	-	-	-
10	120.78	Ar-C	-	-	-
11	36.87	CH	1.81	1H	T
12	65.25	CH-O	3.72	1H	Q
13	31.11	CH_2	1.63	2H	T
14	49.63	CH	1.85	1H	T
15	86.20	CH	2.20	1H	Q
16	172.38	C=O	-	-	-
17	25.32	CH_2	1.95	2H	Q
18	26.46	CH_2	2.00	2H	T
19	79.11	CH-O	3.82	1H	M
20	64.22	C	-	-	-
21	90.12	O-CH_3	3.51	3H	S
22	12.01	CH_3	1.25	3H	S
23	12.01	CH_3	1.25	3H	S
24	12.01	CH_3	1.25	3H	S
25	92.21	O-CH_3	3.71	3H	S
26	90.36	CH-O	3.82	1H	D
27	56.37	C	-	-	-
28	12.01	CH_3	1.25	3H	S
29	12.01	CH_3	1.25	3H	S
30	12.01	CH_3	1.25	3H	S
31	75.01	C≡C	-	-	-
32	75.01	C≡C	-	-	-
33	98.44	CH-O	3.94	1H	S
34	72.06	C	-	-	-
35	90.12	O-CH_3	3.51	3H	S
36	12.01	CH_3	1.25	3H	S
37	12.01	CH_3	1.25	3H	S
38	12.01	CH_3	1.25	3H	S
1’,2’,3’,4’,5’ & 6’	98.44	$C_6H_{11}O_6$	4.69	11H	Ddp

s = singlet, d = doublet, t = triplet, q = quartet, ddp = doublet of doublet of pentet and m = multiplet

Another type of proton detected in the spectrum is a methine proton (CH), which is presented in the positions H-3, H-11, and H-14 at δ 1.71 ppm, δ 1.81 ppm and δ 1.85 ppm with a singlet (s), a triplet (t) and a triplet (t) multiplicities respectively. They are also, in electron-rich environments. H-12 (CH-O), H-15 (CH), H-19 (CH-O), H-26 (CH-O) and H-33 (CH-O), with signals at δ 3.72 ppm, δ 2.20 ppm, δ 3.82 ppm, δ 3.82 ppm and δ 3.94 ppm and their multiplicities as quartet (q), quartet (q), triplet (t), doublet (d) and singlet (s) respectively. They are de-shielded inductively and anisotropically. Another proton detected in the spectrum is the

aryl proton (Ar-H) presented in position H-7 with a resonance frequency signal at δ 7.31 ppm and a singlet (s) multiplicity. It is de- shielded by the anisotropic effect of the benzene ring. An overlap represented in positions H- 1^1, H-2, H-3, H-4, H-5, and H-6^1 at a signal of δ 4.69 ppm with a doublet of doublet of pentet (DDP) multiplicity indicated that a sugar moiety is attached to the aglycone unit. ^{13}C NMR spectrum of compound revealed different kinds of carbon atoms in compound. These carbon atoms include; methyl (CH_3) carbon atoms, which are represented in positions C-22, C-23, C-24, C-28, C-29, C-30, C-36, C-37, and C-38 with a chemical shift value of δ 12.01 ppm, which depicted that the carbons are equivalent and are in the same environment, this is analogous to tertbutyl group. C-21 and C-35 (CH_3O) at δ 90.12 ppm, are methoxy carbon atoms that are in the same environment and are de-shielded by inductive effects. C-25 at δ 92.21 ppm is another methoxy carbon that is de-shielded by the inductive effect of an oxygen atom [38-40].

Another type of carbon atom revealed by the spectrum is methylene (CH_2) carbon atoms which are represented in positions C-1, in an electron-dense environment with a resonance frequency at δ 30.63 ppm. C-2 at δ 23.18 ppm is another methylene carbon atom in an electron-dense environment, including C-4 with a signal at δ 36.87 ppm, shielded by electron density, C-13 at δ 31.11 ppm, also shielded by electron density, C-17 at δ 25.32 ppm, also shielded by electron density and C-18, which resonates at δ 26.46 ppm, also in an electron-rich environment (28). Another type of carbon atom revealed by the spectrum is methine (CH) carbon atoms which are represented in positions C-3 at δ 34.15 ppm, shielded by electron density, C-11 at δ 36.87 ppm, shielded, C-12 (CH-O) at δ 65.25 ppm, de-shielded by inductive effect of an electronegative atom, oxygen, which tends to pull electrons close to itself, C-14 at δ 49.63 ppm is de-shielded by anisotropy, because, it is bonded to a carbon atom bearing a triple bond. C-15 at δ 86.20 ppm, is highly de-shielded by the anisotropic effect of C=O. Again, C-19 (CH-O) is an oxymethine that resonates at a high-frequency signal of δ 79.11 ppm, and it is de-shielded by the inductive effect of an oxygen atom. Also, C-26 at δ 90.36 ppm, is highly de-shielded by the inductive effect of an oxygen atom, and C-33 (CH-O) at δ 99.44 ppm, is also, highly de-shielded by anisotropy of the triple bond [28].

Another type of carbon atoms revealed were aryl carbons (Ar-C), which are the aromatic carbon atoms and are represented in positions C-5, C-6, C-9, and C-10 at a resonance frequency signal of δ 120.78 ppm, which indicated that they are in the same environment and are de-shielded by the anisotropic effect of the benzene ring. C-7 (Aromatic proton-H) at δ 109.55 ppm is de-shielded by anisotropy and C-8 (Ar-C-O) at δ 135.03 ppm is de-shielded by both anisotropic and inductive effects [31]. Another type of carbon revealed is quaternary carbons which are represented in positions C-20 (C) at δ 64.22 and it is de-shielded. C-31 and C-32 (C≡C) are substituted alkyne carbon atoms that resonated at δ 75.01 ppm and are de-shielded by anisotropy. C-34 at 72.06 ppm, is also de-shielded by 2-J coupling to a methoxy carbon and 2-J coupling to a carbon atom bearing a triple bond, that is, it is de-shielded by both inductive and anisotropic effects. Another type of carbon revealed by the spectrum is a carbonyl group (C=O) which resonated at δ 172.38 ppm. It is highly de-shielded by anisotropic effect. A cluster of peaks at δ 98.44 ppm depicted the presence of a sugar moiety bonded to the aglycone unit [31].

3.5 HSQC of Compound

The Fig. 4 shows the HSQC spectrum of compound displaying various correlations between different carbon atoms and the protons that are bonded to them.

The HSQC spectrum of compound revealed a cross peak at δ 56.37 ppm which correlated with the proton at δ 1.25 ppm with a singlet multiplicity, and this corresponded to C-27 bonded to three methyl protons at H-28, H-29, and H-30. Another cross peak at δ 64.22 ppm which also correlated with the same proton at δ 1.25 ppm with a singlet (s) multiplicity, corresponded to C-20, bonded to H-22, H-23, and H-24. Another cross peak was observed at δ 72.06 ppm which also correlated with the same proton at 1.25 ppm and this also, corresponded to C-34 and H-36, H-37 and H-38 that were bonded to it. This implies that the protons are chemically and magnetically equivalent and this depicted that three tert-butyl groups were attached to three different quaternary carbons.

The cross peak at δ 56.37 ppm also correlated with the proton at δ 4.69 ppm and δ 6.00 ppm which corresponded to the sugar moiety.

The compound is a glucocorticoid steroid with the IUPAC nomenclature, Cis-3-(2,2-dimethyl-26-(((2S,3R,4S,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl)-8-methoxy-15-((S)-3-methoxy-4,4-dimethylpentyl)-14-(3-methoxy-4,4-dimethylpent-1-yn-1-yl)-1,2,3,4,11,12,13,14,15,15a-octahydrophenanthro[2,1-b]furan-16(10H)-one, with a molecular formular of $C_{44}H_{68}O_{11}$. It is a glycosteriod because, there is an attachment of a sugar moiety to the aglycone unit, through a β -glycosidic linkage between the anomeric carbon, C-1^1 of the sugar, and the C-26 of the aglycone unit.

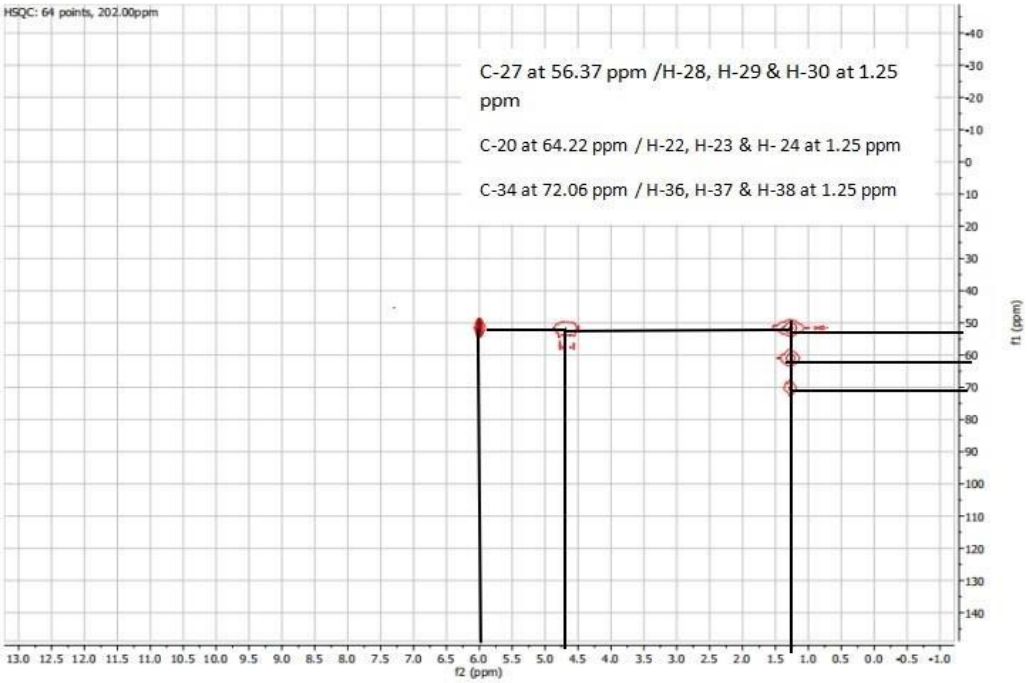

Fig. 4. HSQC spectrum of compound

STRUCTURE OF COMPOUND 1 (E5)

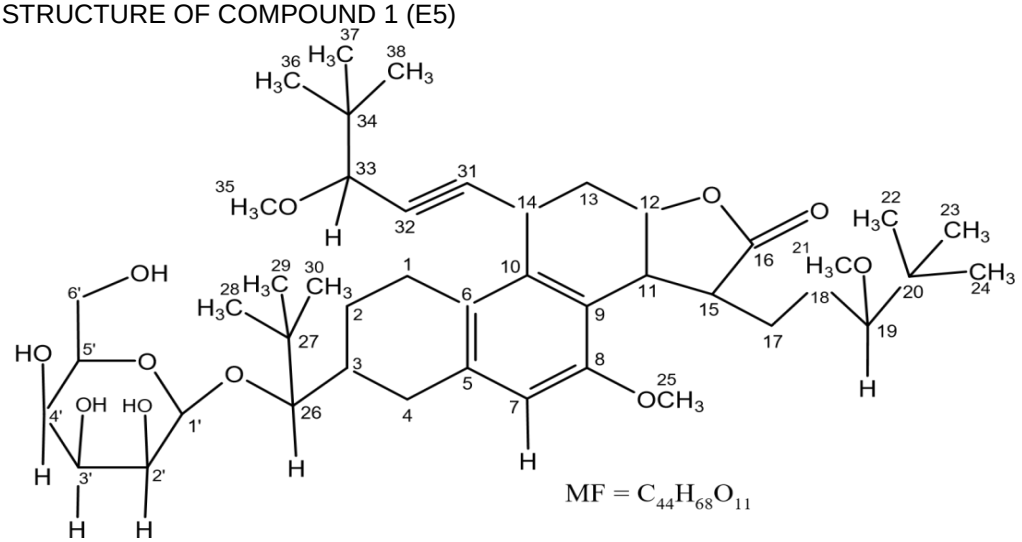

Fig. 5. Proposed structure of compound

Cis-3-(2,2-dimethyl-26-(((2S,3R,4S,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)butan-2-yl)- 8methoxy-15-((S)-3-methoxy-4,4-dimethylpentyl)-14-(3-methoxy-4,4-dimethylpent-1-yn-1-yl)-1,2,3,4,11,12,13,14,15, 15a- octahydrophenanthro[2,1-b]furan-16(10H)-one

4. CONCLUSION

The study of the leaf of *R. vomitoria* yielded a glucocortcoidal steriod, which can serve as immunomodulatory agent and immunosuppresants in acute chronic conditions. Inflammation is the root cause of asthma, therefore the leaves of the plant is useful for the treatment of inflammatory diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abo KA, Ogunleye VO, Ashidi JS. Antimicrobial poteintial of Spondias mombin, Croton zambesicus and Zygotritonia crocea. Journal of Pharmacological Research. 1991; 5(13):494- 497.
2. Liu RH. Potential Synergy of Phytochemicals in Cancer Prevention Mechanism of Action. Journal of Nutrition. 2004; 4(3):47-95.
3. Nweze EL, Okafor JL, Njoku O. Antimicrobial Activities of Methanolic extracts of Trume guineas (Scchumn and Thorn) and Morinda lucinda used in Nigerian Herbal Medicinal practice. Journal of Biological Research and Biotechnology. 2004; 2(1):34-46.
4. Doughari JH, Human IS, Bennade S, Ndakidemi PA. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin-producing bacteria. Journal of Medicinal Plants Research. 2009; 3(11):839-848.
5. Usman I, Hussain M, Imran A, Afzaal M, Saeed F, Javed M, Afzal A, Ashfaq I, Al Jbawi E, Shamaail AS. Traditional and Innovative Approaches for the Extraction of Bioactive Compounds. Int. J. Food Prop. 2022; 25(1):1215–1233. DOI: 10.1080/10942912.2022.2074030.
6. Etukudo I. Conventional and traditional uses of plants. 2003; 13-16.
7. Ehiagbonare EJ. Regeneration of Rauwolfia vomitoria. Afr. J. Biotechnol. 2004; 6(8):979-981.
8. Odugbemi T. A Textbook of Medicinal Plants from Nigeria: Lagos, University of LagosPress; 2008.
9. Kutalek R, Prinz A. African Medicinal Plants in Yaniv Z and U. Bachrach (eds) Handbook of medicinal plants: New Delhi, CBS publishers; 2007.
10. Dieudonne ML, Brice IK, Emma FB, Judith D. Fertility enhancing effects of aqueous extract of Rauwolfia vomitoria on reproductive functions of male rats. Journal of Experimental and Integrative Medicine. 2013; 4(1):43-49.
11. Iwu IC, Chinonye C, Samuel IC, Obiagwu I, Uchenna OL, Ukaoma AA, Chijioke Okere M, Ezekoye MO. Chemical and medicinal properties of Rauwolfia vomitoria (Afzel) harvested from South Eastern Nigeria. Asian Journal of Chemical Sciences. 2021;10(4):56-71.
12. Amita P, Shalini T. Concept of standardization, extraction, and pre-phytochemical screening strategies for the herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014; 2(5):115-119.
13. Sofowora AE. Medicinal plant and traditional medicine in Africa. 3rd edition, Spectrum Books Limited, Ibadan. 2008; 199-205.
14. Boham BA, Kocipai AC. Flavonoids and condensed tannins from leaves of Hawaina Vaccinium and V. Calycinium.Pacific Sci. 1994; 48:458-463.
15. Emmanuel M.T., Kwabena M.B., William K.A., Regina A., Kofi B.O., Mabel D.T., Felicia A.K., Alfred, A.A., Veronique P.B., Alexander K.N. In Vitro Assessment of Anthelmintic Activities of Rauwolfia vomitoria (Apocynaceae) Stem Bark and Roots against Parasitic Stages of Schistosoma mansoni and Cytotoxic Study. Journal of Parasitology Research; 2017. Article ID 2583969,11 pages.
16. Chanet A., Milenkovic D., Manach C., Mazur A., Morand C. Citrus flavanones: what is their role in cardiovascular protection? Journal of agricultural and food chemistry. 2012; 60(36):8809-8822.
17. Fortunato LR, Alves CDF, Teixeira MM, Rogerio AP. Quercetin: a flavonoid with the potential to treat asthma. Brazilian Journal of Pharmaceutical Sciences. 2012; 48(4):589-599.
18. Asoegwu SN, Ohanyere SO, Kanu OP, Iwueke CN. Physical properties of African

oil bean seed (Pentaclethra macrophylla) Agricultural Engineering international. The CIGR E- Journal, iii. ; 2006.

19. Oboh G. Antioxidative Potential of Ocimum Gratissimum and Ocimum Canum Leaf Polyphenols and Protective Effects on Some pro-oxidants Induced Lipid Peroxidation in Rat Brain: An in Vitro Study. American. J. Food.Technol. 2008;3:325–334.
DOI: 10.3923/ajft.2008.325.334.
20. Rao AV, Gurfinkel DM. The Bioactivity of Saponins: Triterpenoid and Steroidal Glycosides. Drug Metabol Drug Interact. 2000;17:211–235,
Available:https://doi.org/10.1515/dmdi.2000.17.1- 4.211.
21. Sparg SG, Light ME, Van Staden J. Biological activities and distribution of plant saponins. Journal of Ethnopharmacology. 2004;94:219-243.
22. Kandeel M, Balaha M, Inagaki N. Kitade Y. Current and future asthma therapies. Drugs of today. 2013;49(5):325-339.
23. Ghosh D. Tannins from foods to combat diseases. International journal of pharmaceutical sciences review and research. 2015;4(5):40–44.
24. Møller C, Hansen SH, Cornett C. Characterization of tannin-containing herbal drugs by HPLC. Phytochem. Anal. 2009;20:231–239.
25. Laurie M, Erika V, Sandy A, Isabelle M, Yuting M. Science Translational Medicine. 2012;4:143-199.
26. Wagner KH, Elmadfa I. Biological relevance of terpenoids: an overview focusing on mono-, di and tetraterpenes. Annals of Nutrition Metabolism. 2003;47:e106.
27. Xu R, Fazio GC, Matsuda SPT. On the origins of triterpenoid skeletal diversity. Phytochemistry. 2004;65:261-291.
28. Bruice PY. Organic Chemistry. University of California, Santa Barbara. 6th ed. PrenticeHall. 2011;501-622.
29. Pattnaik S, Dash D, Samantaray DP. Evaluation of IAA and PHAs Production by Chromium Resistant Bacterial Isolates. Journal of Experimental Biology and Agricultural Sciences. 2018;6(3):466 – 471.
30. Egbucha JN, Echeme JO, Igwe OU, Otuokere IE. Isolation and Characterization of a Novel Tertiary Substituted Amine from the Leaves of Sarcophrynium brachystachys (Benth.) K Schum. Tropical Journal of Natural Product Research (TJNPR). 2023;7(7):3502–3507.
31. Morrison RT, Boyd RN. Organic Chemistry. 6th ed. Published by Asoke K. Ghosh, Prentice-Hall of India Pvt. Ltd., M-97, Connaught Circus, New Delhi. 2005;585-642.
32. Kwekowe CG, Johnbull EO, Otuokere IE. Isolation and Characterization of Secondary Metabolite from the Stem Bark Extract of Allophylus africanus Beauv (Sapindaceae). Journal of Chemical Society of Nigeria. 2021;46(2):0382–0392.
33. Iwu MM, Gbodossou E. The Role of Traditional Medicine. Lancet. 2000;356: S3.
DOI: 10.1016/S0140-6736(00)91989-5.
34. Zahnit W, Smara O, Bechki L, Souici CB, Messaoudi M, Benchikha N, Larkem I, Awuc hi CG, Sawicka B, Simal-Gandara J. Phytochemical Profiling, Mineral Elements, and Biological Activities of Artemisia Campestris L. Grown in Algeria. Horticulturae. 2022;8(10):914.
DOI: 10.3390/horticulturae8100914.
35. Egbucha JN, Echeme JO, Igwe OU, Otuokere IE. Isolation and Characterization of a Novel Tertiary Substituted Amine from the Leaves of Sarcophrynium brachystachys (Benth.) K Schum. Tropical Journal of Natural Product Research (TJNPR). 2023;7(7):3502–3507.
36. Ekarika CJ, Emmanuel IE, Anwanabasi EU, Ubong SB. Analysis of the Constituents of Rauwolfia vomitoria Ethanol Root Extract using GC-MS. World Journal of Innovative Research. 2020; 2(9):32 -34.
37. Rajakumar N, Shivanna MB. Traditional herbal medicinal knowledge in Sagar taluk of Shimoga district, Karnataka, India. Indian Journal of Natural Products and Resources. 2010;1(1):102-108.
38. Rex JRS, Muthukumar NMSA, Selvakumar PM. Phytochemicals as a potential source for anti-microbial, anti-oxidant and wound healing—A review. MOJ Bioorg. Org. Chem. 2018;2:1.
39. Usunobu U. A Review of Some African Medicinal Plants, International Journal of Pharmaand Biosciences. 2012;3(4):1-11.

40. Tavs AA, Ogechi KO, Freddy OA, Gerald IE. Antisickling and Toxicological Evaluation of the Leaves of Rauwolfia vomitoria Afzel (Apocynaceae). Journal of Science and Practice of Pharmacy. 2014; 1(1):11-15.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115202