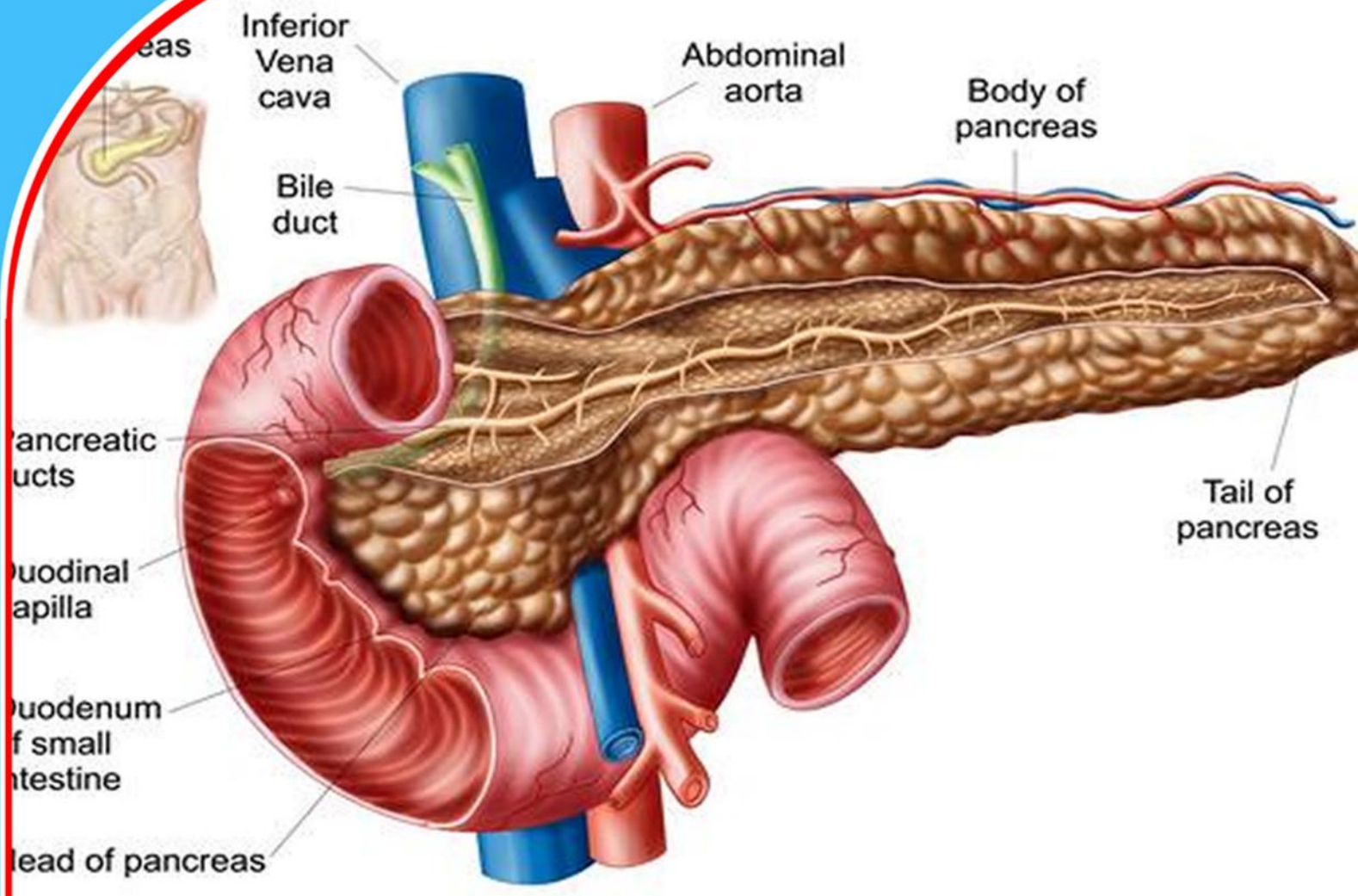
European Journal of
Biology
(EJB)



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AJP

European Journal of Biology
ISSN 2709-5886 (Online)
Vol.8, Issue 2, pp 1 -14, 2023

www.ajpojournals.org

Preliminary Qualitative Phytochemical Screening and Isolation of Sitosterol and Sitostinone from *Dieffenbachia Seguine* Stem Bark

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Article history
Submitted 11.12.2022 Revised Version Received 19.12.2023 Accepted 20.12.2023

Abstract

Purpose: Plant kingdom has long served as a prolific source of useful drugs, foods additives, flavouring agents, colourants, binders, and lubricants. *Dieffenbachia seguine* (Areceae is claimed to have many folklore uses. Primarily it is used as an ornamental and sometimes employed for medicinal and other miscellaneous uses). The stem and root extracts have been described as a narcotic, a gastric and kidney irritant and historically used as arrow poisons, pounded. It is used as a choleretic, female aphrodisiac, and contraceptive and to treat dropsy, gout, dysmenorrhea, impotence, and sterility. The present study aimed at carrying out phytochemical screening and purification of extracts of the plant.

Materials and Methods: Powdered sample of the plant was extracted successively with hexane, ethylacetate and methanol.

Findings: Preliminary qualitative phytochemical screening showed presence of flavonoids, tannins, alkaloids, glycosides, anthraquinones, steroids and triterpenoids. Saponins was however, was not detected in any of the extracts. The hexane and ethyl acetate extracts (2 g) were subjected to silica gel (70-230 mesh) column chromatography and similar fractions were pulled together through thin layer chromatography analysis to obtain fractions Ds-1, Ds-2 and Ds-3 which were subjected to nuclear magnetic resonance (NMR) spectroscopy.

Implications to Theory, Practice and Policy: Fractions Ds-1, Ds-2 and Ds-3 were identified as octadecanoic acid, sitosterol and β-sitostenone, respectively, based on analysis of NMR spectra and comparison with reported data. *Dieffenbachia seguine* extracts are rich sources of phytochemicals which can be purified for more compounds.

Keywords: *Dieffenbachia Seguine, Phytochemical, Chromatography, NMR Spectroscopy*

1.0 INTRODUCTION

Historically, natural products have formed the basis of medicines and many of the compounds that are pharmaceutically and medicinally important are derived from natural sources (Zwenger and Basu, 2008). Plant kingdom has long served as a prolific source of useful drugs, foods additives, flavouring agents, colourants, binders, and lubricants. Plants are known to have medicinal and therapeutic importance in the prevention and treatment of diseases and ailments such as malaria, hypertension and diabetes mellitus and this knowledge has been passed down from one generation to another either orally or in writing (Tor-Anyiin *et al*., 2016).

The dumb cane plant *Dieffenbachia seguine* (Jacq.) Schott is an exotic plant in Nigeria that is believed to originate from the neotropics where it occurs as an understorey herb of the primary tropical rain forests in its native habitat in Mexico and elsewhere in the new world (Cuartas-Hernandez and Nunez-Farfan, 2006). The plant is native to tropical America and the West Indies, especially Costa-Rica and Colombia but presently it can be found in many tropical and subtropical climates, but its toxicity was known as early as the seventeenth century (Blessing *et al*., 2009).

Dieffenbachia seguine (Jacq.) Schott belongs to the angiosperm family Areceae, subfamily Aroideae and tribe, Dieffenbachieae. Over ten synonyms are associated with it, among which are: *Arum seguine* Jacq, *Caladium maculatum*, *D. amoena*, *D. baraquiniana*, *D. exotica* hort., *D. lineata* and *D. picta*. It is commonly called dumb cane, dumbplant, mother-in-law-plant, zebra plant or spotted dieffenbachia. An erect perennial herb, *D. seguine* can reach up to 1-2 m high or more with a rather stout caudex that is conspicuously ringed stem and accumbent, the lower part rooting at the nodes. The leaves are 15-40 cm long, simple, alternate, with narrowly oblong-ovate blades with an acute-acuminate apex that sometimes appears as a short, fine and almost thread-like apiculum. Ornamentation on the leaf surface is formed by patterns of many irregular yellowish or cream-green splotches or white spots and flecks.

The leafbase is shallowly cordate with rounded lobes, midrib is prominent and lateral veins curved-ascending. The petiole is 30 cm long, grooved and winged from the base to beyond the halfway point. It rarely flowers in Nigeria, but has been reported to flower infrequently during the year elsewhere; flowers are many, borne tightly packed in a cylindrical spadix usually 14-17 cm long with an oblong-lanceolate, persistent, greenish or white spathe 15-25 cm long. The fruit is a globose red-orange berry but infrequently formed in cultivation (Aigbokhan, and Agbontaen, 2012.). Bassey and Akpanumun (2009) listed *D. picta* (a synonym for *D. seguine*) among the six invasive plants in Akwa Ibom State, Nigeria and reported that it was becoming invasive in Ikot Uso Akpan Wildlife Sanctuary at Itu.

The plant is claimed to have many folklore uses. Primarily it is used as an ornamental and sometimes employed for medicinal and other miscellaneous uses (Bosch *et al*., 2002). The stem and root extracts have been described as a narcotic, a gastric and kidney irritant and historically used as arrow poisons, pounded *D. picta* is used as a revulsive for rheumatism, rashes and skin itches in west Indian (Dutta, 2002). It is used as a choleretic, female aphrodisiac, and contraceptive and to treat dropsy, gout, dysmenorrhea, impotence, and sterility (Ayensu, 1978).

D. seguine was among the components of Simon's Zombie poison preparation (Inglis, 2010). Zombification is a religious practice related to voodoo from perspective of voodoo, zombies are created by witchcraft, an essentially magical phenomenon. The zombie poison powder was

controlled by Haitian secret societies with root in West Africa. The poison was and still is used as a form of sanction for those who violated the codes of the society (Littlewood and Douyon, 1997).

In the Bahamas it is told that the culprit of a crime robbed the lips of an eye witness to his crime with a Dieffenbachia stem. The witness was not able to testify in court and the accused man was acquitted (Dutta, 2002).

Decoction of *D. seguine* were said to be used in Gabonese traditional medicine for the treatment of haemorrhoids, paralysis and ulcerative incurable wounds (Line-Edwige *et al*., 2009). Oloyode *et al.* (2012) investigated the antioxidant (DPPH free radical scavenging activity) properties of the leaves and stem of hexane, ethyl acetate and water fractions of *D. Seguine* as compared to butylated hydroxyanisole (BHA) α- tocopherol and ascorbic acid, which are known antioxidants. The result showed that fractions from D *seguine* had only moderate activities as free radical scavengers when compared with ascorbic acid, butylated hydroxyanisole (BHA) and α-tocopherol. The low scavenging activity of D. *seguine* could be linked to the absence of secondary plant products like flavonoids (Cushnie *et al.,* 2005).

Ganiyat *et al.* (2011) demonstrated that *Dieffenbachia seguine* exhibited appreciable antimicrobial activity against ten tested microorganisms namely: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonellae typhi*, *Candida albicans*, *Candida krusei*, *Aspergillus niger* and *Penicllium notatum.* Antimicrobial activities of extracts from leaves and stem of *D. Seguine* using agar well diffusion method was investigated by Oloyede *et al.* (2012) It was observed that all the tested samples possessed antimicrobial activities which make them to have broad spectrum of both gram positive and gram negative and the fungi used. They showed inhibition in the range of 10 to 24 mm for all the fractions at 200 mg/ml, the Inhibition capacities of the fractionated extracts are considerably very close to that of the positive controls. Oloyede and co-workers also demonstrated that the stem bark fraction (hexane extract) was more active on two bacterial namely: *E. coli* with diameter of inhibition of 2 mm *P. aeruginosa* with diameter of 20 mm but active on all the fungi.

Line-Edwige *et al*. (2009) investigated antiproliferative effects of alcoholic extracts of leaves of *D. seguine* on human colon cancer cells. It was further observed that leaves extracts of *D. seguine* containing saponosides possessed very weak antiproliferative activity on caco-2 cells with IC_{50} higher than 50 mg/mL. This result did not show the importance of saponosides in the fight against cancer. Plants drugs are primary source of naturally occurring fertility regulating agents because of their little or no side effects. Newman *et al.* (2000) in a study on therapeutic activities of herbal drugs reported *D. seguine* as one of the plants that have anti-fertility activities (Sehgal, 2003). A rat study performed by Madus and Kochi substantiates sterility after 40-90 days of feeding male mice and 30-50 days of feeding female mice with dieffenbachia juice or injecting it subcutaneously (De Pasquale *et al*., 1984).

Araceae contain crystals of calcium oxalate, which are often cited causing the intense irritation experienced when handling or consuming the raw plant tissue of many genera in the family. This supposition is contradicted by the fact that irritation generally is not produced by properly cooked plants, the crystals remain after heating. Other compounds must therefore be involved which cause this reaction. Whether irritation is caused by enzymes or crystals, many genera of Araceae are included in the lists of poisonous plants (Dan *et al*., 2004). The poisonous compounds in *D.*

Seguine might likely be present in the non-polar fraction of the plant. However, the ingestion of the polar fraction may not be harmful to the human body because of its non-toxicity

Typically, the plant causes painful swelling of the mouth and throat after chewing on it, but some symptoms of oedema have resulted from only placing the stem on the lips or coming in contact with the juice. Because it is such a common home or office plant, it is frequently involved in accidental ingestions and exposures. It most commonly affects children and unsuspecting adults who mistake the *Dieffenbachia* for an edible plant. Toxic exposure to *Dieffenbachia* is through three routes: ocular, dermal, and oral. Ocular toxicity causes severe pain, chemosis, photophobia, blepharospasm, lacrimation, corneal abrasion, and kerato conjunctivitis (Orge *et al*., 2010).

Ingestion of the leaves or stems of *Dieffenbachia* species results in rapid development of local mouth and throat irritation (Spoerke and Smolinske, 1990). Redness, swelling and burning pain of the tongue and mucous membranes are noted initially, swelling may rarely become severe enough to produce obstruction and respiratory compromise. Profuse salivation and dysphagia are often present. In severe cases, impairment or loss of speech may occur, lasting for several days. Painful tongue and buccal bullae and necrosis may persist for a week or longer. (Spoerke and Smolinske, 1990). It is uncommon for plants parts to be swallowed because of immediate pain upon ingestion if swallowed, laryngeal, oesophageal, or gastric oedema and constriction of the glottis, inflammation peaks in an hour and may continue from a few days to two weeks (Pamies *et al*., 1992)

Exposure to the juice has resulted in immediate intense pain, photophobia, followed by eyelid, blepharospasm, watery eyes, conjunctival chemosis, and corneal abrasions. Needle-like crystals are frequently visible on corneal epithelium (Spoerke and Smolinske, 1990), when brought into the eye, the sap can cause injury of the cornea, and all parts of the plants are very poisonous when ingested (Cumpston *et al*., 2003). Exposure to cut stems or juice may produce local inflammation (Spoerke and Smolinke, 1990). Through an intensive literature review, Pedaci *et al*. (1999) ranked the types of toxicity caused by *Dieffenbachia seguine*. The most common were oral irritation, dermalpain, vomiting, erythema, throat irritation, and dermal oedema.

The toxicological effect of *Dieffenbachia Seguine* Schott on Wister albino rats was investigated by Ajuru *et al*. (2018). Single oral doses of the leaf extract at the concentrations of 0, 4000, 6000, and 8000 mg/kg/day was administered to albino rats to determine the acute toxic effects and the median lethal dose (LD_{50}) in rats. For hematological study, blood samples were collected by cardiac puncture and analyzed. The kidney, liver, and heart organs were collected, sectioned and viewed under the microscope for histological study. The results revealed no significant changes in the control group but in the treatment groups, several changes such as fast and laboured breathing, blurred vision and death were observed.

Hematological study showed reduced blood level in the treatment group. Morphological observation of the organs including the liver showed alteration and paled coloration compared to the control group. Histopathological examination showed distorted, congested and focal necrosis of the liver and renal tubules. The results obtained revealed that the ethanol extract of *D. Seguine* has high toxicity profile regarding haematological and histological parameters in albino rats. Despite its toxicity and medicinal value, literature information is scanty on the stem bark of *Dieffenbachia seguine* and general evaluation of the plant phytochemistry.

2.0 MATERIALS AND METHODS

Collection of Preparation of Plant Material

The stem bark of *D. seguine* was harvested from a garden in Judges Quarters, Makurdi in the month of July 2017. The botanical identification of the plant was done by Mr. Mark Uleh, at the Department of Forestry and Wildlife, University of Agriculture, Makurdi and a voucher specimen of number 901 was deposited at the Herbarium. Stem bark of *D. Seguine* was cleaned, chopped into pieces and air dried at room temperature for a period of 21 days. The dry bark was ground into powder. The powdered plant material was weighed and used for extraction.

Extraction of Plant Materials

The powdered plant material (150 g) was extracted with hexane (700 mL) in a 1 litre glass jar by soaking 150 g of the powdered plant into 700 mL of hexane for 48 hours with intermittent manual agitation. The procedure was repeated with ethyl acetate and methanol. The extracts were filtered through Whatman number 1 Filter Paper. The filtrate were coded as DSH (Hexane extract), DSE (ethyl acetate extract) and DSM (methanol extract) and allowed to evaporate at room temperature.

Qualitative Phytochemical Screening

The crude extracts of hexane, ethyl acetate and methanol from stem bark of *Dieffenbachia seguine* were screened for the presence of secondary metabolites using Standard Procedures as described by Tor-Anyiin *et al.* (2016) and Sofowora (2008).

Tests for Flavonoids

Lead acetate test: Extracts of hexane, ethyl acetate and methanol were treated with few drops of lead acetate solution.

Alkaline reagent test: 2 mL of 2.0 % NaOH mixture was mixed with aqueous extracts of hexane, ethyl acetate and methanol and 2 drops of dilute HCl was added to the mixture

Test for Alkaloids

Extracts of hexane, ethyl acetate and methanol were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test: filtrates were treated with Mayer's reagent (potassium Mecuric Iodide)

Wagner's test: filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).

Dragendroff's test: filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide)

Hager's test: filtrates were treated with Hager's reagent (saturated

Tests for Glycosides

Liebermann's est: 2.0 mL of acetic acid and 2 ml of chloroform were added to the hexane, ethyl acetate and methanol extracts. The mixture was then cooled and concentrated sulphuric acid was added to it.

Keller-Kiliani test: A solution of glacial acetic acid (4.0 mL) with 1 drop of 2.0 % $FeCl_3$ mixture was mixed with the 10 mL aqueous hexane, ethyl acetate and methanol extracts and 1 mL of concentrated H_2SO_4 added to it.

Salkowski's test: Concentrated sulphuric acid (2 mL) was added to hexane, ethyl acetate and methanol extracts.

Test for Steroids and Terpenoids

Salkowki's test: Chloroform (2 mL) was added to the extract (0.5 g), few drops of concentrated sulphuric acid were carefully added down the side of the test tube to form a lower layer. The production of a reddish brown ring at the interface would indicate the presence of steroids.

Liebermann-burchard's test: Chloroform (2 mL) was added to the extract (0.5 g) and filtered. Acetic anhydride (2 mL) were added to the filtrate and shaken, few drops of concentrated sulphuric acid was carefully added down the side of the test tube to form lower layer.

Test for Saponins

Ten (10) mL each of the hexane, ethyl acetate and methanol extracts were dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponins contents in the sample. The powdered plant material (2.0 g) was boiled with distilled water in a test tube in boiling water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously

Test for Tannins

Lead sub acetate test: The extract was dissolved in water (1 mL) and lead sub acetate solution (1 mL) was added. The production of coloured precipitates indicates the presence of tannins.

Ferric chloride test: To the extract (0.2 g) was stirred with water (5 mL) and filtered. Few drops of ferric chloride was added to the filtrates, a blue or bluish black precipitates showed the presence of hydrolysable tannins while formation of green precipitates would indicate the presence of condensed tannins

Test for Anthraquinones

Ten (10) mL of benzene was added in 6 g of the plant powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 mL of 10 % ammonia solution was added to the filtrate and shaken vigorously for 30 seconds

Column Chromatography

The ethyl acetate extract (2 g) were subjected to silica gel (230-400 mesh ASTM) column chromatography. Silica gel (50 g) was mixed with hexane to form a homogenous suspension/slurry and stirred using a glass rod to remove bubbles. The silica gel slurry was then poured into a glass column. The plant sample to be loaded on the column was prepared by dissolving 2 g of the extract in 100 mL of methanol. To the solution, 5 g of silica gel was added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature and crushed to obtain a free flowing powder which was then loaded onto column. Gradient column elution was carried out using silica gel stationary phase with increasing solvent polarity in the order hexane: ethyl acetate (90: 10), (80: 20), (70:30), (60:40), and (50:50). A total of 85 fractions were collected in 10 mL vials.

Thin Layer Chromatography (TLC)

The concentrated fractions collected from the column chromatography were subjected to thin layer chromatography (TLC). A spot of each fraction was carefully applied onto a TLC plate (coated with silica). After about five minutes, the plate was dipped in a suitable solvent which allowed the compound in the spot to move upwards by capillary attraction. The following solvent systems were used; 9:1 v/v hexane in ethyl acetate was used for 1-20, 8:2 for fractions 21-50 and 7:3 for fractions 51-80. The plate was then removed from the solvent and left to dry. The plates were sprayed with 20 % sulphuric acid in methanol and charred (heated). The retention factor R_f values of all the spots were determined by the following formula:

$$R_f = \frac{Distance\ travelled\ by\ the\ spot}{distance\ travelled\ by\ the\ solvent}$$

Based on similarities in R_f values, the 80 fractions were combined into three sub-fractions, labelled Ds-1, Ds-2 and Ds-3.

Test for Class of Compounds

Fractions Ds-2 and Ds-3 were dissolved in 5 mL of acetic anhydride, boiled and cooled concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the inter-phase.

Determination of Melting Point

Few crystals each of Ds-1, Ds-2 and Ds-3 were introduced separately into a capillary tube (10-15 cm), and sealed at one end. The capillary which contained the sample and a thermometer were then suspended so that they were heated over a Bunsen burner. The temperature when the substance starts melting (t_1) and the temperature when the substance melted completely (t_2) were noted. The average of the two readings (t_1 and t_2) was taken as the melting point of the substance.

Spectroscopic Analysis

^{1}HNMR Spectroscopic analysis of Ds-1, Ds-2 and Ds-3 were carried out using 400 MHz Spectrometer (Bruker) at the Strathclyde Institute of Pharmacy and Biomedical Sciences Laboratory, University of Strathclyde, Glasgow, Scotland UK.

3.0 FINDINGS

Table 1: Phytochemical Screening

Class of Compounds	Methanol Extract	EtOAc Extract	Hexane Extract
Flavonoids	+	+	-
Tannins	+	+	-
Glycosides	+	-	-
Anthraquinone	+	+	-
Alkaloids	+	+	+
Saponins	-	-	-
Steroids/ triterpenoids	+	+	+

Key

+ = Positive - = Negative EtOAc = Ethyl Acetate

Phytochemical Screening

Results of preliminary phytochemical screening showed that methanol and ethyl acetate extracts showed presence of flavonoids, tannins, anthraquinones and alkaloids, steroids and terpenes. The hexane extract indicated the presence of alkaloids only. This result agrees with the report by Oloyede *et al.* (2012) except that they found no anthraquinones and glycosides.

Physical Characteristics of Extracts and Isolated Compound

Methanol extract had the highest yield followed by ethyl acetate extract. This suggests that most of the compounds present in *D. Seguine* are polar.

Ethyl acetate extract had orange colour. Thin layer chromatography of fractions from the extract showed that fractions 21 – 37, 38 – 47, 67 – 85, had similar R_f values. They were then combined and labelled as DS-1, DS-2 and DS-3 respectively. They were dried by allowing the solvents to evaporate.

Characterisation of DS-2 as Sitosterol

DS-2 was a colourless needle-like crystal with melting point of 138-139 ^oC. Its TLC plate showed a pinkspot upon spraying with 20 % sulphuric acid and heating. The R_f value of was determined (0.50; hexane: acetyl acetate 9:1). Liberman Buchard's test for steroids (Talukdar *et al.*, 2010), was also positive. This indicated that DS-2 could be a steroid/triterpenoid. The proton NMR of DS-2(^{1}H-NMR 400MHz($CHCl_3$) showed signals at δ_H 5.34 ppm, δ_H3.66 ppm, δ_H2.28 ppm, δ_H2.02 ppm, δ_H1.84 ppm, δ_H1.65 ppm, δ_H1.63 ppm, δ_H1.60 ppm, δ_H1.56 ppm, δ_H 1.51 ppm, δ_H 1.50 ppm,δ_H1.49 ppm, δ_H1.48 ppm, δ_H1.47 ppm, δ_H1.45 ppm, δ_H1.40 ppm, δ_H1.04 ppm, δ_H 1.10 ppm, δ_H 1.02 ppm, δ_H0.88ppm, δ_H 0.85 ppm, δ_H 0.80 ppm,δ_H 0.69 ppm,δ_H 0.68 ppm.

^{1}H – NMR Spectra for DS-2 recorded in $CHCl_3$ showed signal at δ_H 5.34 ppm, δ_H 3.66 ppm, δ_H 2.28 ppm, δ_H 2.02 ppm, δ_H 1.57 ppm, δ_H 1.31 ppm δ_H 1.02 ppm, δ_H 0.84 ppm and δ_H 0.69 ppm.DS – 2 showed multiplets at δ_H5.34 ppm and δ_H3.66 ppm. These signals as reported by Shimbe *et al.* (2016) are characteristic of olefinic protons. Other signals at δ_H 0.69 ppm and 1.02 ppm corresponds to signals typical for angular methyl protons (Pateh *et al.*, 2009). A comparison of the ^{1}H NMR data of DS-2 with other reports by Tor- Anyiin *et al.* (2016), Mouffok *et al.* (2012), Saeidnia *et al.* (2014), Arora and Kalia (2013) and Sen *et al.* (2012) reveals that DS-2 is β-sitosterol. Table 4 showed the ^{1}H –NMR data of DS-2 compared to reports in literature.

Table 2: Comparison of Experimental and Literature ^{1}H NMR Data for DS-2

Position	Group	Experimental Data	Shimbe *et al.* (2016)	Mouffok *et al.* (2012)	Pateh *et al.* (2009)
1	CH_2	1.31	1.47	1.50	1.47
2	CH_2	1.57	1.56	1.56	1.56
3	CH_2	3.66	3.52	3.58	3.52
4	CH_2	2.28	2.18	2.30	2.28
5	C	-	-	-	-
6	CH	5.34	5.34	5.40	5.34
7	CH_2	2.02	2.03	1.50	2.03
8	CH	1.66	1.67	2.03	1.67
9	CH	1.48	1.48	0.98	1.48
10	C	-	-	-	-
11	CH_2	1.50	1.52	1.55	1.52
12	CH_2	1.49	1.49	2.06	1.49
13	C	-	-	-	-
14	CH	1.45	1.52	1.04	1.50
15	CH_2	1.60	1.61	1.63	1.60
16	CH_2	1.84	1.84	1.89	1.84
17	CH	1.40	1.49	1.16	1.49
18	CH_3	0.68	0.67	0.74	0.68
19	CH_3	1.02	1.02	1.06	1.02
21	CH_3	1.01	0.94	0.93	0.94
22	CH_2	0.88	0.88	1.07	0.88
23	CH_2	1.04	1.04	1.21	1.04
24	CH	1.60	1.49	0.97	1.50
25	CH	1.65	1.65	1.71	1.65
26	CH_3	0.81	0.83	0.88	0.83
27	CH_3	0.84	0.85	0.87	0.85
28	CH_2	1.04	1.04	1.31	1.04
29	CH_3	*	0.88	0.89	0.88
30	OH	*	2.00	-	2.00

* = overlapping signals

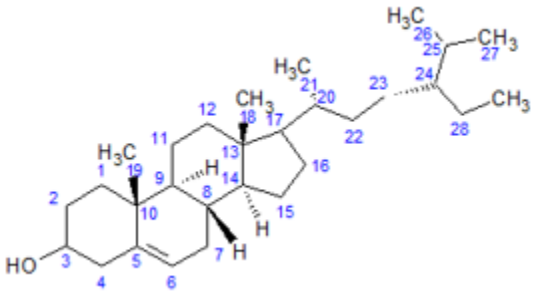

Figure 1: Structure of β-Sitosterol

Characterization of DS-3 as β–Sitostenone (stigmast-4-ene-3-one)

Ds -3 was isolated as a white crystal with melting point 96 – 98 ^{o}C. Its TLC plate showed a single orange spot upon spraying with 20 % sulphuric acid. The R_f value (0.39; hexane: acetyl acetate; 8:2). Furthermore, the presence of a red violet colour upon treatment of the compound with 2.5 mL of acetic anhydride and 2.5 mL of chloroform followed by addition of sulphuric acid indicated its triterpenoid nature.

The ^{1}H NMR of DS-3 (400MHz($CDCl_3$)) was recorded asδ_H 5.72(d, *J*=1.8H_3, 1H) 2.39 – 2.35 (m 2H), 2.33(dd, *J*=7.8, 3.6 4H, 2.30 – 2.24 (M, 4H) 2.00(ddd, *J*=10.2, 70, 24 H_3, 6H), 1.86 – 1.81(M, 2H), 1.05 – 0.99(M, 4H), 1.52(d, *J*=3.5H_3, 2H), 1.18 – 1.13 (M, 7H), 1.05 – 0.99(M, 5H), 0.90(dd *J*=5.6, 3.1H_3), 0.87 – 0.79(M, 21H), 0.73 – 0.67 (M, 4H).

^{1}H–NMR Spectra for DS – 2 gave signals at δ5.72, 2.37, 2.33, 2.27, 2.00, 1.83, 1.65, 1.52, 1.48, 1.15, 1.01, 0.90, 0.90, 0.83 and 0.70.

The ^{1}H–NMR data for β-sitostenone reported by Parachayasttiko *et al*. (2009) had signals at δ5.74, 1.19, 0.93, 0.85, 0.84 and 0.72. The ^{1}H–NMR data for β-sitostenone as reported by Ryan (2001) gave signals at δ5.33, 3-5, 2.27, 1.00, 0.93, 0.93, 0.86, 0.84, 0.82, 0.69. A careful comparison of DS -2 ^{1}H-NMR data with ^{1}H–NMR data published for β-sitostenone by Prachayasttiku *et al.* (2003), Ara *et al.* (2009) and Ryan (2001) showed DS-2 had 4 methyl protons.

The ^{1}H–NMR spectrum of DS-2 exhibit a proton doublet at δ_H 5.72. This according to Ara *et al.* (2009) corresponds to an olefinic proton. According to Prachayasttiku *et al.* (2009), signals observed at δ_H 0.80, 0.83, 0.90 and 1.01 corresponds to H_{12}, H_{26}, H_{27} and H_{29} respectively which were identified as methyl protons. Ara *et al.* (2001), Prachayasttiku (2009) and Ryan (2001) also have reported on ^{1}H-NMR of sitostenone. These reports agree with DS-2 ^{1}H-NMR data obtained in this work. The similarities between DS-2 ^{1}H-NMR data and ^{1}H-NMR data published for β-sitostenone and the TLC result led to the conclusion that DS-2 is β-sitostenone an Isomer of sitosterone.

Characteristic peaks and coupling constants were found to be identical to that of β-sitostenone as previously reported by Ara *et al.* (2009). Sitostenone have been isolated from several plant species including *Birsera serrate* (Ara *et al.*, 2009), *Spilanthenac mellamirr* (Prachayasisttikul *et al.*, 2009) *Collectrichom gloeosporoides* (Carvalho *et al.*, 2016) and *Draceana angustifolia* (Ryan, 2001). Sitostenone is a triterpenoid with diverse activities such as significant hypoglycemic (Alexander–lindo *et al.*, 2007), antiarrhythmic (Hotta *et al.*, 2003) and antitubercular (Salude *et al.*, 2002) activities.

Table 3: Comparison of Experimental and Literature Data for DS-3

Position	Group	Ex. Data	Paracchayasttiko *et al.* (2009)	Ara *et al.* (2009)	Ryan *et al.* (2001)
1	CH_2	1.48	-	-	-
2	CH_2	1.52	-	-	-
3	CH_2	2.27	-	-	3.50
4	CH_2	2.37	-	-	2.27
5	C	-	-	-	-
6	CH	5.72	5.74	5.74	5.33
7	CH_2	2.00	-	-	-
8	CH	0.70	0.71	0.72	0.93
9	CH	-	-	-	-
10	C	-	-	-	-
11	CH_2	-	-	-	-
12	CH_2	-	-	-	-
13	C	-	-	-	-
14	CH	-	-	-	-
15	CH_2	-	-	-	-
16	CH_2	0.83	0.83	-	0.82
17	CH	-	-	-	-
18	CH_3	-	-	-	-
19	CH_3	-	-	-	-
21	CH_3	-	-	-	-
22	CH_2	-	-	-	-
23	CH_2	-	-	-	-
24	CH	-	-	-	-
25	CH	-	-	-	-
26	CH_3	0.83	-	0.85	0.85
27	CH_3	0.82	-	0.84	0.84
28	CH_2	1.01	1.10	-	-
29	CH_3	0.90	-	0.93	0.93
30	OH	-	-	-	-

Key: Ex. = Experimental

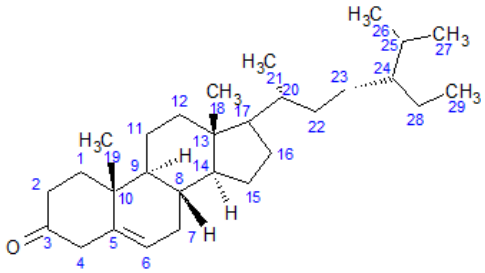

Figure 3: Structure of β-Sitostenone

4.0 CONCLUSION AND RECOMMENDATIONS

The phytochemical screening of *Dieffenbachia seguine* has shown that the stem bark extracts of the plant contains flavonoids, tannins, steroids, terpenoids, cardiac glycosides and alkaloids. Saponins are however not present. These phytochemicals presents are reported to have various medicinal potencies and this confirm the use of *D. seguine* traditionally to treat several ailments as reported by Ayensu (1978), Dutta (2002) and Line-Edwige *et al.* (2009).

Therefore, this plant is good source of traditional medicine and can be used to screen for several kinds compounds in drug discovery process. The chemical compounds isolated from this plant, sitostenone, sitosterol and octadecanoic acid could be synergistically responsible for treatment of diseases as claimed by traditional healers. On the basis of chromatographic and spectroscopic techniques, octadecanoic acid, sitosterol and sitostenone have been isolated, characterized and reported for the first time from the stem bark of *Dieffenbachia seguine.*

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