Antisickling and Antioxidant Effects of Diodia scandens, Cochlospermum tinctorium and Ficus exasperata Aqueous Extract

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Abstract

In this study, Diodia scandens, Cochlospermum tinctorium and Ficus exasperata Plants were studied because it offer a large range of natural compounds belonging to different classes of phytochemicals.
These molecules possess interesting biological activities which have attracted several researchers to their elucid ation to provide knowledge that will lead to advancement in medicine. Nigeria stands out as the most sickle cell endemic country in Africa, this is due to either ignorance or poor standard management. A large percentage of t he affected ones have no access to required blood transfusion, rely on traditional phytomedicine to prevent sickling and alleviate painful crisis. Patients with sickle cell disease (SCD) have inherited genes which lead to the presence of sickle cells (drepanocytes) in their blood. The specific genetic mutation that results in sickle hemoglobin involves a substitution of thymine for adenine (from GAG to GTG) on the sixth codon of the genetic sequence. This leads to the coding of valine rather than glutamate on the sixth position of the hemoglobin beta chain.
This study was designed to identify the bioactive constituents present in Diodia scandens and its possibility of synergism for antisickling and antioxidant activities, which could be beneficial to SCD patients when administered.

Key word: *Hemoglobin, Antisickling, Thymine, Genes, Synergism*

Introduction

Plants have been used for treatment of many diseas es since ancient time, people of all continents especially Africa have this old tradition. Medicinal plants have been shown to have genuine uses and about 80 percent of the rural population depend on them as primary health care (Akinyemi, 2000). Despite remarkable progress in synthetic organic m edicinal product of twentieth century over 25 perce nt of prescribed medicine in industrialized countries are derived directly or indirectly from plants (New man *et al*., 2000). Plants offer a large range of natur al compounds belonging to different classes of ph ytochemicals. These molecules possess interesting biological activities which have attracted several re searchers to their elucidation to provide knowledge that will lead to advancement in medicine (Zabri *et al*., 2008). The practice of traditional medicine was described as Herbalism or Botanical medicine (Evans, 2002). Two-third of the world population (mainly in the developing countries) relies entirely on such traditional medical therapies as their primary form of health care (Sumner, 2000). The bioactive i ngredients that have the therapeutic activity in plant s used in traditional practice are mostly unidentified (Adebanjo et al,. 1 983).

Examples of active principles include: anthraquinon es, flavonoids, glycosides, saponins, tannins, morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different diseases (Chevalier, 2000) . Sickle cell disease (SCD) is a devastating condition that is caused by an autosomal recessive inherited hemoglobinopathy which results in the vaso-occlusive phenomena and hemolysis. The severities of the complications that occur with this disorder varied, but overall mortalit y is increased and life expectancy decreased when c ompared to health individuals (Jones, 1961). Painfu l vaso-occlusive events are the most common complication experienced by both children and adul ts with sickle cell disease and there are few treatment options to prevent the development of these events (Oduola, *et al*., 2006). Most are managed with traditional supportive care measures (i.e. aggressive hydration, anti-inflammatory and narcotic analgesics) that have not changed in decades and which are adequately met by the current World Health Organization (WHO, 2021). Sickle c ell disease belongs to a family of disease known as hemoglobinopathies. It was first 'discovered' in the United States in 1910, when a physician Dr James Herrick observed sickled shape red cells in the blo od of a West Indies patient with pain and anaemia. I t is the commonest genetic disorder here in Nigeria affecting about 4 million Nigerians at prevalence o f 2% at birth while over 40 million individuals have sickle cell trait, Nigeria accounts for 75% of new born(infant) SCD in Africa (Kassim et al., 2015).

Biochemically, Sickle cell diseases CD occurs due t o a non conservative substitution of a polar glutam ate (Glu) by non polar valine (Val) in an invariant r egion, the sixth position of Hb β chain subunit (GA G/GTG; Glu (E), Val (V); rs334) (Väliaho, et al., 2 015).

Replacement of this single non polar amino acid val ine results in a biochemical difference that leads to formation of a sticky patch on the surface of the β chains. The sticky patch is observed on both the ox ygenated (R Form) and deoxygenated (T Form) of HbS. This distort folding and binding pattern of the Hb molecule, due to altered properties. Other know n mechanism of polymerization of Hb in SCD invo lves nucleation, which is due to the aggregation of HbS molecules. At low oxygen level (hypoxia), deoxyhemoglobin S polymerizes inside the red blood cells (RBC). Due to oxygen deprivation in the RBC, a critical aggregate of Hb polymer is formed that damages the cellular membrane , promoting aggregation of cellular proteins, stopping the flow of blood in the narrow capillaries and leading to lo

calized oxygen deprivation (anoxia) (Väliaho, *et al*., 2015).

Materials and Methods

Plant collection and authentication

The plant was collected in Damaturu, Yobe State, Nigeria. The plant was taken to the herbarium of the department of biology, Yobe State University for identification and authentication.

Extraction

The plants was cut into pieces, air dried and ground ed. 100g of the grounded stem bark was then susp ended in 1L of water and allowed to stand for 24 h ours at room temperature 28ºC. The mixture were then filtered using whatman's filter paper. Additional water was added to the residue for total extraction. The filtrate was evaporated at 50ºC using water bath.

Polarity guided partitioning

The crude extract was solvent partitioned using n-hexane (De, *et al*,. 2009). The hexane extract and polar residue obtained was used for the antisickling study. The fractions obtained was dried in a dish under fan, after which they were then placed in a sample bottles and stored in a refrigerator at 4ºC until required. The percentage yields of the fraction was calculated using the formula:

$$\text{Yield of fraction } \% = \frac{\text{weight of fraction}}{\text{weight of water extract}} \text{ x } 100$$

Red Blood cells sickling reversal test

Ability of the plants bioactive component to revers e the sickling state of RBC was performed using m ethod described by (Paulin *et al*,. 2013). The blood sample was washed in phosphate buffered saline (P BS; pH 7.4) twice by centrifugation at 1200g. In a c lean Eppendorf tube, 100µL of the washed red bloo d cells and 100µL of freshly prepared 2% sodium metabisulfite was added and incubated for two hour s at 37ºC. Then 100 µL of the anti-sickling agent were added and incubated for another two hours at 37ºC. Then 10 µL of the incubated cells is dilute d 100 times, a drop placed on a slide and covered using cover slip, then the cells was counted using a n Olympus Microscope at 40× magnification. The c ells are classified normal or sickle by observing thei r shapes. Biconcave or disc like shapes is considere d normal while elongated, star like or wrinkle shape s is considered sickled. A control test was then *performed* by replacing anti-sickling agent with 100 µL of PBS. The experiment will be repeated five times. The percentage sickled cells was calculated u sing the formula:

$$\text{Percentage Sickling } (\%) = \frac{\text{Number of sickled cells}}{\text{Total number of counted cells}} \times 100$$

Alkaloids

1cm^3 of 1% HCl was added to 3 cm^3 of the extracts in a test tube. The mixture was heated for 20 minute s, cooled and filtered. The filtrate will be used in th e following tests: 2 drops of Wagner's reagent will be added to 1 cm^3 of the extract. A reddish brown p recipitate indicates the presence of alkaloid.

Tannins

1 cm^3 of freshly prepared 10% KOH will be added to 1 cm^{3}of the extracts. A dirty white precipitate indicates the presence of tannins.

Phenolics

Twodrops of 5% $FeCl_3$ will be added to 1cm^3 of the extracts in a test tube. A greenish precipitate indica tes the presence of phenolics.

Glycosides

To 10cm^3 of 50% H_2SO_4 will be added to 1cm^3 of the e xtracts, the mixture will be heated in boiling water for 15 minutes. 10cm^3 of Fehling's solution will be added and the mixture boiled. A brick red precipita te indicates.

Saponins

A
2cm^3 of the extract will be measured in test tube an
d vigorously shaken for 2 minutes. Frothing

Indicates the presence of saponins.

Flavonoids

To
1m^3 of 10% NaOH will be added to 3 cm^3 of the ext
racts. A yellow colour indicates the presence

of flavonoids.

Steroids

Salakowsti test: 5 drops of concentrated H_2SO_4 will be added to 1 cm^3 of the extracts. Red colouration i
ndicates the presence of steroids.

Phlobatannins

A
1cm^3 of the extracts wasF added to 1% HCl. A red precipitate indicates the presence of phlobatannin.

Triterpenes

Five
drops of acetic anhydride were added 1 cm^3 of the extracts. A drop of concentrated H_2SO_4 will
be added and the mixture will be steamed for 1 hou
r and neutralized with NaOH followed by the addit
ion of chloroform. A blue green colour indicates th
e presence of triterpenes.

Phytosterols

Phytosterols (Finar 1986): Liberman burchard's tes
t: 50mg were dissolved in a 2ml acetic anhydride.
To this, a
drop of conc.H_2SO_4 was added slowly along the sides of the test tube.
An array of colour changes showed the presence of phytosterols.

Terpenoids

To
5ml of aqueous extract of the sample were mixed w
ith 2ml of CHCl3 in a test tube in which
3ml of conc. H_2SO_4
was carefully added to the mixture to form a layer. An interface with a reddish

Brown coloration is formed which indicates that terpenoids constituent is present.

Amino acid (Yasuma and Ichikawa 1953): 2 drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) will be added to 2ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

Antioxidant activity assay
Determination of reducing power:

Reducing power will be determined by the method described by Oyaizu *et al.*(1986). The sample in 1
ml of methanol at various concentrations was mixe
d with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5ml, 1%), and the mixture was incubated at 50°C for 20 min. Next, 5ml of trichloroaceticacid (10%) was added to the reaction mixture, which was then centrifuged at 3000 RPM for 10 minutes. The upper layer of the solution (5ml) will be mixed with distilled water (5ml) and ferricchloride (1ml, 1%), and the absorbance was measured at 700 nm. A str
onger absorbance will indicate increased reducing p
ower.

**Ferric Reducing Antioxidant Power (FRAP) Ass
ay:**

The ability to reduce ferric ions was measured using the method described by Benzie and Strain (1996). The FRAP reagent was generated by mixin
g 300 mM sodium acetate buffer (pH 3.6), 10.0mM (tripyridyl 10:1:1 in volume. Samples at different concentrations (100,200,300,400

and 500 µg/ml) was then be added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593nm was measured. Fresh working solutions of $FeSO_4$ was used for calibratio n. The antioxidant capacity based on the ability to

reduce ferric ions of sample was calculated from th e linear calibration curve and expressed as mmol F eSO_4 equivalents per gram of sample (DW).

Determination of peroxide (H_2O_2) radical scaven ging activity:

The hydrogen peroxide scavenging assay was carrie d out. A solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The extract at different concentrations in 3.4 ml phosphate buffer was added to 0.6 ml of H_2O_2 s olution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm.

$$H_2O_2 \text{ scavenging activity } (\%) = \frac{A0 - A1}{A0} \times 100$$

Where A_0 is the absorbance of the control, and A1 i s the absorbance of the sample.

Determination of nitric oxide (NO) radical scave nging activity:

The hydrogen peroxide scavenging assay was carrie d out following the procedure of

Ruch *et al*. (1989). A solution of H_2O_2 (43 mM) wa s prepared in phosphate buffer (0.1 M, pH 7.4). The extract at different concentrations in 3.4 ml phosphate buffer was added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM). The absorbance value of the react ion mixture will be recorded at 230 nm.

$$\text{NO scavenging activity } (\%) = \frac{A0 - A1}{A0} \times 100$$

Where A0 is the absorbance of the control, and A1i s the absorbance of the sample.

Determination of hydroxyl radical (-OH) scavenging activity:

The scavenging activity of extracts on hydroxyl rad ical was measured according to method previously described method (Mensor *et al*., 2001). In 1.5 mL of each diluted extract, 60 µL of $FeCl_3$ (1 mM), 90 µL of 1 ,1 0 Phenanthroline (1 mM), 2.4 ml of 0.2 M phosphate buffer, pH 7.8 and 150 µL of H_2O_2 (0 .17 M) was added respectively. The mixture will then be homogenized and incubated at room temperature for 5 min. The absorbance was read at 560 nm against the blank. The percentage of the rad ical scavenging activity of each extract will be calc ulated from the equation below:

Percentage of radical scavenging activity = $\frac{\text{OD control} - \text{OD sample}}{\text{OD}} \times 100$.

Statistical Analysis

Each test was perform in triplicate and the results w as expressed as mean standard error of mean

(SEM). The Kruskal Wallis non parametric test foll owed by a post hoc Dunnet T3 ($p < 0.05$) will be used to analyze the antioxidant capacity, total phenols content as well as the radical scavenging activity of each extract and percentage of sickling of different partition extracts. The software Ms Excel for Windows 7 was used for statistical analysis.

RESULTS AND DISCUSSION

Phytochemical screening of the various plants crude aqueous extract is presented in the results below.

TABLE 1: Sickling Reversal Effects of DS, CT and FE Extracts

SAMPLE	CT (Aq)	DS (Aq)	Fel" (Aq)
Saponin	+	+	+
Tanin	+	-	+
Phenolic	+	-	+
Steroids	+	+	+
Coumarin	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Terpenoids	+	+	+
Triterpenes	-	+	+
Anthocyanin	-	-	-
Phlobatanin	-	-	-
Amino ACIDS	-	-	-
Alkaloids	-	+	-

Table 2: Antioxidant Activity of the crude aqueous DS, CT and FE Extracts

Partitioned Extract	*%Sickling DS New students*	*%Sickling CT Graduating students*	*Ch%Sickling FE ange*
Control	51.98±6.38a	51.98±6.38a	51.98±6.38a
HSE	33.19±2.44	33.19±2.44	33.19±2.44
Crude	41.49±0.310b	30.95 ± 15.21b	41.13±0.58b
N-hexane	25.85±1.45d	19.57±2.00c	7.29±1.35c
Polar	37.66±2.18c	5.27±0.50 d	57.14±5.02d

Values are mean of three replicates ± SEM. Values with different superscript down the same column are significantly different ($p < 0.05$)

Table 3: Compounds in aqueous crude extract PubChem compound data base (https://pubchem.ncbi.nlm .nih.gov)

S/N	Phytochemical compounds	Pubchem ID	Retention time (RT)in Min	Area%	Chemical formula
1	2,3-dihydroxypropylester(Z)-,9-Octadecenoicacid	9 2 2 6	5153	45.74	$C_{13}H_{26}O_4$
2	cis-Vaccenic acid	5282761	5.153	45.74	$C_{18}H_{34}O_2$
3	9-Octadecenoicacid	9 6 5	5.153	45.74	$C_{18}H_{34}O_2$
4	methylesterHexadecanoic acid	102118220	23.572	33.45	$C_{18}H_{34}O$
5	Methylester (Z)-9-Octadecenoicacid	6441996	27.003	17.62	$C_{21}H_{39}O_5$
6	methylester12-Octadecenoicacid	5364503	27.003	17.62	$C_{19}H_{36}O_2$

The GCMS screening of aqueous crude extracts revealed the presence of 2,3- dihydroxypropyl ester (Z)-9-Octadecenoic acid, Cis-vaccenic acid,9- Octadecenoic acid, MethylesterHexadecenoic acid, Methyl ester (Z)-9-Octadecenoic acid, Methylester 1 2-Octadecenoic acid as the most abundant phytoconstituents.

Table 4: Some Compounds in *Ficus exasperate* Aqueous Crude Extract

Phytochemical compounds	Pubchem ID	Retention time (RT) in min	Area %	Chemical structure
Methyl ester	553681	23.557	60.03	$C_{23}H_{50}O_4Si_2$
Hexadecanoic acid				

Methyl ester trans-13Octadecenoic acid	5364506	26.985	36.00	$C_{19}H_{36}O_2$
Methylester11 -Octadecenoic acid	91749551	26.985	36.00	$C_{28}H_{60}O_5Si_3$
Methylester(Z) -9-Octadecenoic acid	102024920	26.985	36.00	$C_{20}H_{38}O_2$

The Table above shows the phytochemical compounds in *Ficus exasperata* aqueous crude extract, their retention time (RT) in minutes and percentage Xcal which are; Methyl ester Hexadecanoic acid, Methyl ester trans-1 3-Octadecenoic acid, Methyl ester 1 1 -Octadecenoic acid and Methyl ester (Z)-9-Octadecenoic acid. Their RT are; 23.557, 26.985, 26.985 and 26.985 respectively. Their percentage area is; 60.03, 36.00, 36.00 and 36.00 respectively.

Table 5: Chemical structures of phytochemical compounds in *Cochlospermum tinctorium*

S/N	Phytochemical compound	Pubchem ID	Retention time (RT) in Min	Area %	Chemical structure
1	methylesterHexadecanoic acid	553681	23.570	59.2 1	$C_{23}H_{50}O_4Si_2$

2	14-methyl-,methylester Pentadecanoic acid	21205	23.570	59.2 1	$C_{17}H_{34}O_2$
3	methylester11-Octadecenoic acid	91749551	26.998	36.6 7	$C_{28}H_{60}O_5Si_3$
4	Methylestertrans- 13Octadecenoicacid	6161490	26.998	36.6 7	$C_{18}H_{34}O_2$
5	methylester(Z)- ,9Octadecenoicacid	6441996	26.998	36.6 7	$C_{28}H_{39}BO_5$

The Table 5 above indicates chemical structures, retention time (min) and percentage area of the major phytochemical constituents identified in *Cochlospermum tintorium* aqueous crude extract using GCMS. Methyl ester Hexadecanoic acid, 1 4-methyl-methyl ester Pentadecanoic acid, methyl ester 1 1 -Octadecenoic acid, Methyl ester trans-1 3-Octadecenoic acid, and methyl ester (Z)-,9 Octadecenoic acid. Their retention time were 23.570, 23.570, 26.988, 26.888, 26.988 respectively. Percentage
area are; 59.21, 59.21 , 36.67, 36.67, 36.67 respectively.

Discussion

Qualitative determinations were carried out on the extracts for the presence of saponin, steroids, coumarins, flavonoids, glycosides, terpenoids, triterpenes, and alkaloids. Tanin, phenolic, anthocyanins, phlobatannins and amino acids were not detected as presented in Table 1 above, this result is similar to the findings of other researcher's work. Cowan (1 999) enumerated and analyzed various phytochemical constituents found in medicinal plants that are used as antibacterial, antiviral, antifungal or antiparasitic and they include: quinones, phenols, flavonoids, tannins, terpenoids, alkaloids, polyphenols and others. All these bioactive compounds are responsible for preventing oxidation (antioxidants), antisickling and some other therapeutic properties.

Flavonoids (a natural substances) mainly presents in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine. More than 4000 varieties of flavonoids have been identified (Kumar and Pandey, 201 3). The various classes of flavonoids (flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols and anthocyanidins) differ in the level of oxidation. They generally occur in plants as glycosylated derivatives. All type of these flavonoids has capability to act as antioxidants. The flavones and catechins are very important and powerful flavonoids for protecting the body against reactive oxygen species (ROS). Flavonoids also

chelate metal ion results removal of causal factor for the development of free radicals (Kumar and Pandey, 201 3).

Around 1 6,000 alkaloids are known, found in many plant families (*Solanaceae* and *Apocynaceae* where 60-70% of these species accumulate alkaloids) in which 1 0,000 alkaloids are known minimally (20-30% of all plant species layup alkaloids). Plants having alkaloids can be used as dyes, spices, drugs, antiaging and antiviral perspective. These drugs are employed as relaxants of skeletal muscles during surgery to control convulsions. Specifically, the alkaloid interferes with the activity of acetylcholine at the surface where it functions, thereby blocking the neuromuscular junction (Dewick, 2002).

Massive group of glycosides called saponins are widely dispersed in higher plants. A property having surface activity distinguishes it from other glycosides. Shaking with water, the saponins form colloidal solutions with some foam. Saponins are main ingredient of many plant drugs and folk medicines, and also are amenable for various pharmacological characters (Bruneton, 1 995).

For the pharmacological discovery of novel drugs, the primary essential information regarding the chemical constituents is generally provided by the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for different extracts of Diodiascandens showed the presence of several groups of bioactives molecules such as saponin, glycosides and alkaloids. Since the structure and chemical composition of plant extracts affect the ability of permeability, infiltration and solubilization of groups of the bioactives compounds by the solvent. The treatment of erythrocytes with metabisulphite (2%) showed a significant augmentation of sickling. Thus, sodium metabisulphite creates hypoxic conditions for red blood cells leading to the loss of the morphology and sickled erythrocytes. In vitro deoxygenation of RBC by sodium metabisulphite caused progressive aggregation andpolymerization of the individual hemoglobin molecules (Chikezie, 2011). The process of gelation (polymerization) of hemoglobin molecules increases the formation of sickling cells. The sickle cell hemoglobin (HbS) is a product of a defective genetic code of hemoglobin molecule. The sickling cells treated with extracts decreased as well as the naïve red blood cells used in the reversibility. The activity of the extracts could be due to the presence of some bioactive compounds they possess. The phytochemical screening of these extracts revealed compounds such as flavonoids, alkaloids, saponins. The anti-sickling activity could be linked to their ability either to inhibit in vitro polymerization of haemoglobin or to some structural modification linked to the environment of haemoglobin by the extracts (Bienchi et al., 2007). Studies demonstrated that antioxidant molecules were found to be potent inhibitors of sickle cell haemoglobin polymerization, and equally improved the oxidant status of sickle erythrocytes (Imaga et al., 2012).

The relation between antiradical and antioxidant activities of the extracts and their antisickling activity could be explained by the ability of these extracts to give hydrogen or electron atom to the iron molecule of haemoglobin (Kasi et al., 2008). Antioxidants (scavengers of free radicals) are believed to be major components of the anti-sickling properties (Tatum and Chow 1 996). Plants which demonstrated antioxidant property can be therapeutically useful (kanatt et al., 2007). The study reveals and confirms that Diodia scandens has the phytochemicals like alkaloids, steroids, flavonoids, carbohydrates, saponins, etc. are present in the extract (Table 1). These results are similar to the findings of other researchers' work (Cowan and Rathna Kumar 201 3).

GCMS analysis revealed the presence of phytochemical compounds such as: 2,3-dihydroxypropyl ester (Z) -9-Octadecenoic acid, Cis-vaccenic acid,9- Octadecenoic acid, Methyl ester Z-9-Octadecenoic, MethylesterHexadecenoic

acid, Methyl ester-1 2-octadecenoic acid in large amount (Table 5).

According to result of Visveswari et al. (2013) analysis carried out by GC-MS of the methanolic extracts of whole plant (present study) revealed presence of 1 1 compounds at molecular level viz. Oleic Acid, Phytol, n-hexadecanoic Acid, 9-Octadecenoic acid(Z)-, methyl ester, Hexadecanoic acid, methyl ester, 2- Methoxy-4-vinylphenol, Azetidin-2-one 3,3-dimethyl-4-(1 -aminoethyl), 1 7-Octadecynoic acid, O-Bromoatropine, and Sucrose. All these bioacchtive compounds are responsible for the antibacterial property and other therapeutic uses of the plant, e.g. alkaloids have pharmacological effects and are used as medications. Also, some phytochemicals have antioxidant and anticancer activity.

The sickling reversal effects of the Partitioned extracts of Ficus exasperata. Polar extract had the highest percentage of sickled red blood cells of 57.1 4 ± 5.02 while Nhexane had the lowest percentage of sickled red blood cells of 7.29 ± 1 .35. These shows there were relatively little effect on some cells and high effect on some after incubation for 1 20 minutes. If the cell was not in the characteristic sickle shape or in crenated holly leaf pattern (Gorecki et al., 1999). The polymerization of Hbss erythrocyte is a major event in the pathophysiology of sickle cell disease (Oyewole et al., 2008). Hence, the sickling reversal effect of the partitioned extract of Ficus exasperata could be considered significant in alleviating sickle cell symptoms in patients. The results of phytochemical screening (table 4.4) of the Ficus exasperata leave extract, contains phytochemical constituents that have been reported to have the potentials in improving health status. Saponin, Tanin, Phenolic, Steroids, Coumarin, Flavonoids, Glycosides, Terpenoids, Triterpenes, and Alkanoids were found to be present. Flavonoids (Middleton et al 1 986), Tanins (Harborne 1 998), and Saponins (Beutchet 1 997) have anti-inflammatory properties and have the capacity to bind cations, thereby stabilizing erythrocyte membranes (Oyedapo et al 2004) and reducing frequency of SCA crisis. These properties can help the sickle cell patients to fight against the accompanying severe infections which are usually the principal causes of death.

Alkaloids, steroids, saponins, tannins, terpenoids, flavonoids, and anthocyanins were found in the species studied. These are groups of chemical substances with various biological activities including antibacterial, (Hostettmann et al., 2002), antifungal, anticancer, antioxidant, antimalarial, (Bruneton et al., 2009), antiviral, antidiabetic, and hepatoprotective activities (Mbayo et al., 2019). The presence of polyphenols such as anthocyanins and flavonoids are proof that the species studied contain molecules with the capacity to inhibit sickling in vitro and/or in vivo (Mpiana et al., 2015).

Triterpenes, especially pentacyclic ones, represent secondary metabolites that are widely distributed in the plant kingdom and found in leaves, stem bark, fruits and roots (Jäger et al., 2009). They are frequently the object of phytochemical and pharmacological investigations. These results are similar to the findings of other researchers' work. (Cowan and Rathna Kumar 201 3) Result of (Cowan et al., 2023) shows presence of all these phytochemicals in all the plant extracts of Ficus exasperata. The qualitative phytochemical screening of n-hexane extract revealed the presence of phytochemical compounds such as: Methyl ester Hexadecanoic acid, Methyl ester trans-1 3-Octadecenoic acid, Methyl ester 1 1 -Octadecenoic acid and Methyl ester (Z)-9-Octadecenoic acid. (Table 5).

Present study) revealed presence of Oleic Acid, Phytol, n-hexadecanoic Acid, 9- Octadecenoic acid (z)-, methyl ester, Hexadecanoic acid, methylester. 2-Methoxy-4-vinylphenol, Aptidin.-1-3, 3-dimethy-4-(1, aminoethy), 1,7-Octadecynoic acid, O-Bromoatropine, and Sucrose. All these bioactive

compounds are responsible for the antibacterial property and other therapeutic uses of the plant.

Summary

Plants offer a large range of natural compounds belonging to different classes of phytochemicals. These molecules possess interesting biological activities which have attracted several researchers to their elucidation to provide knowledge that will lead to advancement in medicine (Zabri et al., 2008). Nigeria stands out as the most sickle cell endemic country in Africa, due to either ignorance or poor standard management. A large percentage of the affected do not have access to require blood transfusion, rely on traditional phytomedicine to prevent sickling and alleviate painful crisis (Ameh et al., 2012). Patients with sickle cell disease (SCD) have inherited genes which lead to the presence of sickle cells (drepanocytes) in their blood. The specific genetic mutation that results in sickle hemoglobin involves a substitution of thymine for adenine (from GAG to GTG) on the sixth codon of the genetic sequence. This leads to the coding of valine rather than glutamate on the sixth position of the hemoglobin beta chain (Tatum and Chow 1 996; Valiaho et al., 2015). This study was designed to identify the bioactive constituents present in Diodia scandens and possibility of their synergism for antisickling and antioxidant activities, which could be beneficial when administered to SCD patients.

Conclusion

The N-hexane extract of Diodia scandens demonstrated significant antisickling and antioxidant properties. Therefore, further studies are needed to establish these data in transgenic mice models for human SCD. In addition, the toxicity profiles and bioguided fractionation studies need to be undertaken. Alkaloids, flavonoids, terpenoids, steroids, saponin, glycerides and coumarin are found in the Diodia scandens. Diodia scandens play a vital role in preventing sickling and oxidative.

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