Bioactive Profiling of *Vitex doniana* Leaf and its Effect on Rat Erythrocyte Membrane Stabilization Activity

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ABSTRACT

Bioactive profiling of *V. doniana* leaf and its effect on rat erythrocyte membrane stabilization activity was investigated using standard procedures. Study on the proximate composition vitamins compositions, minerals, phytochemicals (qualitative and quantitative), and gas chromatography–mass spectrometry (GC-MS) analysis made up the bioactive profiling. The leaf sample produced high carbohydrate (49.27±0.12%) and crude fats (18.82±0.01%) proximate constituents. Result of vitamins showed that vitamin E > vitamin B12 > vitamin B6 while vitamin K was the least. Zinc was present (1.76±0.21 mg/100g) while the values for [Calcium/Phosphorus] and [Sodium/Potassium] ratios were higher than critical values.

Qualitative phytochemical constituents of *V. doniana* leaf sample showed that tannins, soluble sugars, flavonoids, terpenoids, phenols, and alkaloids were in high concentrations. The quantitative analysis showed that Terpenoids (49.24±0.32 mg/100g) were the highest against Hydrogen Cyanide (HCN) (0.66±0.14 mg/100g) that was the least. Di-n-octyl phthalate ($C_{24}H_{38}O_4$) with highest retention time of 24.87 min and peak area of 35.84% along with other compounds such as 2,3,5,8-tetramethyldecane ($C_{14}H_{30}$), ethyl ester palmitate ($C_{18}H_{36}O_2$), methyl 14-methylpentadecanoate ($C_{17}H_{34}O_2$), ethyl ester-9-octadecenoate ($C_{20}H_{38}O_2$) and 1-octadecene ($C_{18}H_{36}$) were among the compounds observed with GC-MS analysis of the methanol extract of the leaf sample.

Some of these constituents could be behind the activity of the methanol extract of the leaf sample as observed on membrane stabilization of erythrocyte membrane of rat subjected to heat and hypotonic stresses in this study. This study has shown the bioactive profiling of *Vitex doniana* leaf and its effect on rat erythrocyte membrane stabilization activity.

(Keywords: bioactive profiling, membrane stabilization, traditional medicine, *Vitex doniana* leaf)

INTRODUCTION

The practice of traditional medicine involves the use of plants that are effective against disease causing pathogens (Duru, *et al*., 2012). Such practice aided our ancestors in health-related issues. Based on beliefs which have lasted for years and observations, the practice of traditional medicine could be said to have evolved into what is today known as modern medicine (Aburjai, *et al*., 2007). Natural compounds extracted from plants used in traditional medicine practice or their synthetic forms have been recognized as the basis of modern medicine (Agomuo, *et al*., 2017; Nawrot, *et al*., 2003). This is further revealed with modern-day supplements where some known medicinal plants are refined and packaged into tablets and capsules, which when taken into the system, could cure different aliments in humans.

Nigeria is among the countries of the world where there is high belief in the efficacy of traditional medicine despite the advent of modern pharmaceuticals. The high cost of products of modern medicine, culture, and heritage of the people are among the factors behind the consistent popularity of traditional medicine

paramount with the indigenous people of Nigeria. Also, the traditional medicine practice which was nearly destroyed with the coming of the colonial masters who considered the practice barbaric and highly ineffective has gotten a boost of renewed interest in the continents of North America, Asia, and India (Eke, 1999; Duru, 2011). With the recent twist on increased dependency rate of medicinal plants' products by many people from developed countries (Aburjai, *et al*., 2007; WHO 1991; WHO, 1999), the practice therefore goes on to further discover plants of phytomedicine relevance with bioactive compounds and physiological action against disease causing pathogens (Okigbo and Mmeka, 2006; Okwu, 2005; Sofowora, 1993).

Vitex doniana a Verbenaceae is among such plants which has been earmarked for further studies on phytomedicine potentials. Agbafor and Nwachukwu, (2011); Alabo (1999), and Nnenna, *et al*. (2020) noted that the *V. doniana* plant is known as black plum. The plant is known and addressed locally as "uchakoro " or "utakiri", "ori nla or oriri", and "dinyar" by the Igbos, Yorubas, and Hausa tribes of Nigeria, respectively (Adejumo, *et al*., 2013; Nnenna, *et al*., 2020).

V. doniana is a perennial deciduous tree found in savanna and southern regions of Nigeria where it is used in both management and treatment of diseases (Dauda, *et al*., 2011; Nnenna, *et al*. 2020). The newly grown leaves are eaten as vegetable and are also used as source for food materials. Atawodi (2005) noted that different parts of *V. doniana* are used in ethno-medicine to manage and treat numerous disorders such as microbial infection, cancer, rheumatism, hypertension, and inflammatory diseases. Research studies have reported the anti-diabetic (Nwogo, *et al*., 2013; Odika, 2019), anti-lipidaemic (Nwogo, *et al*., 2013), antimicrobial activities (Nwachukwu and Uzoeto, 2010), antihepatotoxicity (James, *et al*., 2010; Ladeji and Okoye, 1996), anti-hypertensive (Olusola, *et al*., 1996), anti-inflammatory (Iwueke, *et al*., 2006); effects on hematological parameters (Abdulrahman, *et al*., 2010), and antidiarrheal activity (Suleiman and Yusuf, 2008) potential of *V. doniana*.

The erythrocyte constituent of the blood fluid is known to play a crucial role in the circulatory system of the body. It is one of the blood cells that facilitate circulatory and functional stability, which must be assured while on either traditional or modern medicine. The erythrocytes, also known as the red cells, must possess the capacity to undergo passive deformation and have the ability to resist fragmentation to be able to facilitate continuous circulation. Membrane stability therefore defines the maximum deformation extent a membrane of erythrocyte can undergo beyond which it can no longer recover its original or initial shape completely (Joel and Narla, 1986).

Normal membrane stability is associated with erythrocytes circulation without fragmentation whereas decreased stability can result in decreased fragmentation under normal circulation stresses and decreased membrane stability can result in numerous stresses which can culminate in diseases of the heart and circulatory system (Joel and Narla, 1986). Changes in blood glucose, blood pressure, and lipid profile indices have been associated with osmotic stability of the erythrocyte membrane (Teixeira, *et al*., 2019).

There are few studies on *V. doniana* in relation to its effect on erythrocyte membrane stabilization activity. This study investigated this area and examined the bioactive profiling of *V. doniana* leaf and its effect on rat erythrocyte membrane stabilization activity.

MATERIALS AND METHODS

Chemicals

All chemicals in this study used were of analytical grade and were purchase from Rovet Scientific Limited, Benin City, Nigeria.

Collection and Preparation of *V. doniana* Leaf Extract

The plant material was collected from Michael Okpara University of Agriculture, Umudike (MOUAU) in September 2016. The plant was identified by Mr. Ibe of the Forestry Department, MOUAU as *V. doniana*. The plant was deposited in the herbarium of MOUAU as was assigned a voucher specimen number MOUAU/BCH/25552/ AB/NG. The leaves of the plant were collected, cleaned and air dried under shade for three weeks. The dried leaved were then crushed to obtain ground sample that was used for the preparation of extract.

The ground sample was used for the preparation of methanolic extract using the method as following the method as described by Uddin, *et al*. (2014) with some modifications. 120 g of ground sample was mixed with 200 mL of methanol and kept in a shaker at 120 rpm for 48 hours at the temperature of 30 ℃. The extract in liquid methanol was filtered using Whatman No 4-filter paper with cotton wool. The process as described was repeated with residue formed. The extracts were then combined and evaporated using a rotary evaporator under a controlled temperature of temperature of 40 ℃ and pressure of about (100 psi). The extract was then obtained in past form. The extract concentrate was calculated as:

$$\frac{\text{Mass of the extract}}{\text{Mass of powder}} \times 100$$

The obtained extract was then stored in the refrigerator at 4 °C until needed for further studies.

Bioactive Profiling of *V. doniana* Leaf Sample

The plant leaf sample was bioactively profiled with the determination of proximate composition, qualitative and quantitative phytochemical analysis, vitamins, and mineral composition analysis, as well as gas chromatography–mass spectrometry (GC-MS) analysis.

Proximate Composition Analysis *V. doniana* Leaf Sample

The moisture content was determined gravimetrically as described by Kirk and Sawyerr (1990). Total ash, crude fat, and crude fiber were determined using their standard methods as outlined by AOAC (2006). The micro Kjeldahl method as described by Muhammed, *et al*. (2010) by multiplying the nitrogen fraction with factor 6.25 was used in the determination of crude protein. Carbohydrate was estimated using 100 - (%moisture + %ash + %protein + %lipid + %fiber) while Atwater factors of 4, 9, and 4 as reported by Onyeike, *et al*. (1995) was used for estimation of energy value of leaf sample.

Qualitative and Quantitative Phytochemical Analysis *V. doniana* Leaf Sample

The outlined procedures as described by Amadi, *et al.* (2004) were used for qualitative determinations of phytochemicals while alkaloids, phenols, and saponins were quantitatively determined by the methods of Harbone (1973) as described in detail by Obadoni and Ochuko (2001). Flavonoids were quantitative estimated with method of Bohm and Kocipal (1974). Hydrogen cyanide and tannins were quantified with the method of Bradbury, *et al*. (1991) and according to Van-Burden and Robinson (1981), respectively. Soluble protein was determined as described by Lowry, *et al*. (1951) method. The estimation of soluble and reducing sugars was method of Nelson (1994). Non-reducing sugars was estimated as the difference of soluble sugars and reducing sugars. Phenol was quantified with the method as described by Oberlease (2003), while steroids, terpenoids and glycosides were determined with the methods as described by El-Olemyi (1994).

Determinations of Vitamins in *V. doniana* Leaf sample

The spectrophotometric method-based UV inactivation as described by Onwuka (2005) was used in the determination of vitamin A. The methods as described by AOAC (2006) were used in the determination of vitamins A, B1, B2, B3, B6, B9, and B12. The method as described by Barakat, *et al*. (1973) was used to determine vitamin C. Vitamins D and E were determined with the standard procedures of the AOAC (1990).

Mineral Content Analysis of *V.doniana* Leaf Sample

The sample was ashed before the minerals were determined. Two grams of the ground sample were weighed into a clean porcelain crucible and dry ashed at the temperature of 550 ℃ using a muffle furnace. The ashed formed was dissolved in 5.0 mL of a mixture of HNO_3/HCl/H_2O (formed in the ratio of 1:2:3) and gently heated on a hot plate until all the brown fumes disappeared. 5.0 mL of deionized water was added and heated to colorless solution. The colorless solution was then filtered into a 100 mL volumetric flask with the help of Whatman filter paper (No. 42) and was made up to the mark with deionized water.

The obtained digest was then used for atomic absorption spectrophotometric analysis (AAS) (Unicam model 969 atomic absorption

spectrophotometer), to ascertain mineral elements (sodium, potassium, calcium, magnesium, iron, zinc, copper and lead), which were calculated as percentage dry matter in mg/100g (Akubugwo, *et al*. (2020), Shadidi, *et al*. (1999). The method as described by Nahapetin and Bassiri (1975) was used to determine the phosphorus content of the digest.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Methanolic Extract of *V. donian* Leaf Sample

The GC-MS analysis of methanolic extract of *V. doniana* leaf sample was carried out using AOC-20i auto sampler and gas chromatography interface to a mass spectrometer following the method as described by Ezekwe, *et al*. (2021).

Assay of Membrane Stabilizing Activity

The membrane stabilizing activity assay was carried out as previously described (Oyedapo, *et al*., 2004; Sadique, *et al*., 1989). 2% (v/v) bovine erythrocyte suspension while Ibuprofen and Indomethacin were used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 2% (v/v) bovine erythrocyte suspension, 0.0 - 1.0 ml of drugs (standard, extracts/fractions) and final reaction mixtures were made up to 4.5 ml with isosaline. Drugs were omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 56°C for 30 min on a water bath, followed by centrifugation at 5000 rpm on Gallenkamp Bench Centrifuge for 10 min at room temperature. The absorbance of the released heamoglobin was read at 560 nm.

The percentage membrane stability was estimated using the expression:

$$\frac{100 - \{\text{Abs of test drug} - \text{Abs of drug control}\}}{\{\text{Abs of blood control}\}} \times 100$$

Where the blood control represents 100% lysis or zero percent stability.

RESULTS AND DISCUSSION

The of proximate composition of *V. doniana* leaf sample as revealed in Table 1 shows the presence of moisture (9.66±0.14 %), crude fats (18.82±0.01%), crude fiber (5.64±0.12%), ash (3.25±0.01%), crude protein (13.38±0.03%), carbohydrates (49.27±0.12%) and energy value (419.98±0.16 Kcal).

Table 1: Proximate Composition of *V. doniana* Leaf Sample.

Proximate content	*V. doniana*
Moisture (%)	9.66±0.14
Crude fats (%)	18.82±0.01
Crude fiber (%)	5.64±0.12
Ash (%)	3.25±0.01
Crude protein (%)	13.38±0.03
Carbohydrate (%)	49.27±0.12
Energy value (Kcal)	419.98±0.16

Values are mean ± standard deviation of triplicate determinations.

Moisture content varies inversely with shelf life of food materials (Okaka and Okaka, 2005, Olusanya, 2008; Whitney, *et al*., 2007). It also shares a relationship with the suitability of food materials to microbial attack and stability (Nwachukwu, *et al*., 2014). The moisture content of *V. doniana* leaf sample in the present study 9.60% is lower than the 16.66% reported by Vunchi, *et al.* (2011) on *V. doniana* (Black plum) fruit pulp. This could therefore imply a longer shelf life for the leaf sample than the fruit pulp. The low moisture content of the leaf sample could also be indication of less suitability to microbial attack in relation to the fruit pulp of *V. doniana*.

The fat constituents of the *V. doniana* leaf sample in this study is 18.82%. The value is relatively low compared to 34.62% reported by Vunchi, *et al*. (2011) on the fruit pulp of *V. doniana*. All high-fat diets (except for n-3 fatty acids) have shown to result in insulin resistance relative to high carbohydrate diets. Saturated, monounsaturated, and polyunsaturated fats, excluding n-3 fatty acids, have caused insulin resistance when fed as high-fat diets to experimental animals (Hedeskov, *et al*., 1992; Storlien, *et al*., 1996; Storlien, *et al*., 1991; Storlien, *et al*., 1986). The types of fat constituent of the studied leaf samples should be investigated for proper possible deduction in relation to diabetic body.

Dietary fiber has been linked with prevention and management of a range of diseases, including type 2 diabetes as early as the seventies (Trowell, 1975; Trowell, 1973). The observed fiber content (5.64%) of the *V. doniana* leaf sample in this study could be imply a more fiber benefits to the body that the fruit reported to produce only 0.58% of fiber by Vunchi, *et al*. (2011) and 4.50% by Aiwonegbe, *et al*. (2018). Anderson, *et al*. (1998) noted that soluble fiber binds to bile acid, which excretes and eliminates cholesterol from the body. According to Anderson, *et al*. (1998) and Arukwe, *et al*. (2012), dietary fiber may help reduced the risk of cancer, especially colon cancer.

According to Ali (2010), Effiong, *et al*. (2009), and Duru, *et al*.(2019), any plant food that provides about 12% of its caloric value from protein is taken as good protein source. Proteins when synthesized, build up in tissue, replace worn-out tissues, synthesize enzymes, hormones and other body products and could also be used as a source of energy (Okaka and Okaka, 2005). The basic units of proteins known as amino acids are linked by peptide bonds to the giant molecule protein. In recent times, amino acid supplementation is gaining acceptance as an important adjuvant therapy in the treatment of diabetes and its associated complications (Evert and Bouchert, 2013; Wheeler, *et al*., 2010). The crude protein content of *V. donian* leaf sample is 13.38%. Crude protein value (13.38%) of *V. doniana* leaf sample in the present study is higher than 8.24% reported on the fruit of *V. doniana* by Vunchi, *et al*. (2011).

The amount and possibly the type of carbohydrate in a food influence overall glucose control. The total amount of carbohydrate (CHO) consumed has the strongest influence on glycemic response. There is currently inadequate evidence in isocaloric comparison recommending a specific amount of carbohydrates for people with diabetes. It has been reported that no correlation has been made between total carbohydrate and diabetes risk (Bessesen, 2001; Steyn, *et al*. 2004). The carbohydrate content of *V. doniana* leaf sample in the present study (49.27%) is higher than the value (28.40%) reported on *V. doniana* fruit by Vunchi, *et al*. (2011). According to Steyn, *et al*. (2004), a wide range of carbohydrate intakes may be acceptable in terms of achieving a low risk of type 2 diabetes with type and source of carbohydrate being more important than quantity.

The total energy content of the studied sample is 419.98 Kcal/100g. This could mean that the leave of *V. doniana* leaf sample may offer energy to the body when consumed. The observed energy content of the studied leaf sample could be indication that it may offer more energy than *Pleurotus tuberregium* as reported by Ikewuchi and Ikewuch (2008), *Pleurotus ostreatus* as reported by Duru, *et al*. (2019), fruit of *Ficus capensis* as reported by Okoroh, *et al*. (2019), *Pleurotus squarrosulus* as reported by Duru, *et al*. (2017) but could offer lower energy against **"**Mberiagworagwo" traditional food (Amadi, *et al*., 2017).

Vitamins A, B1, B2, B3, B6; B9; B12; C, D, E and K in *V. doniana* leaf sample are 1.87±0.12 ug/g, 0.84±0.14 mg/100g, 0.27±0.02 mg/100g, 0.19±0.04 mg/100g, 0.60±0.13 mg/100g, 0.28±0.19 mg/100g, 0.63±0.20 mg/100g, 0.43±0.03 mg/100g, 0.25±0.06 mg/100g, 0.76±0.10 mg/100g and 0.07±0.02 mg/100g respectively as presented in Table 2.

Table 2: Vitamin Constituents of *V. doniana* Leaf Sample.

Vitamin	*V. doniana*
Vitamin A (µg/g)	1.87±0.12
Vitamin B1 (mg/100g)	0.84±0.14
Vitamin B2 (mg/100g)	0.27±0.02
Vitamin B3 (mg/100g)	0.19±0.04
Vitamin B6 (mg/100g)	0.60±0.13
Vitamin B9 (mg/100g)	0.28±0.19
Vitamin B12 (mg/100g)	0.63±0.20
Vitamin C (mg/100g)	0.43±0.03
Vitamin D (mg/100g)	0.25±0.06
Vitamin E (mg/100g)	0.76±0.10
Vitamin K (mg/100g)	0.07±0.02

Values are mean ± standard deviation of triplicate determinations.

The observed fats soluble vitamins of *V. doniana* leaf sample in the present study are appreciable when compared to the values reported by Vunchi, *et al*. (2011) fruits of *V. doniana* and those of other plants. According to Duru, *et al*. (2012), vitamins become very important in the body when their health implications are considered. Okwu (2005) noted that vitamins are important in the body as their deficiencies adversely affect the metabolism of the body. Just recently, Stefan, *et al*. (2017) suggested that carotenoids improve the insulin producing β-cell´s function. Niacin is active in preventing the disease known as pellagra while deficiency of B2 in the diet results in beriberi (Okwu, 2004).

Neuropathy, the severe damage caused to the nervous system by high blood sugar levels, may be associated with deficiency of vitamin B6, also known as pyridoxine. Vitamin B6 also has a strong role to play in the prevention of diabetes-related complications (Stefan, *et al*., 2017). Vitamin B12 may have a strong role to play when treating diabetic neuropathy.

The enormous roles of vitamin C, chemically known as ascorbic acid, have been reported by numerous authors. Apart from its property as an anti-scurvy vitamin, it is also known to facilitate the transformation of cholesterol into bile acid in the liver (Hunt, *et al*., 1980). Ascorbic acid is needed for the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine, respectively. The hydroxylated forms of these amino acids account the role of the vitamin in maintaining normal connective tissue and wound healing, which is a problem in diabetes condition (Okwu, 2005). Ascorbate enhances the reduction of Fe^{3+} to Fe^{2+}, and encourages its dietary absorption (Okwu, 2005). Ascorbate ability to functions as antioxidant, also prevents, or minimizes the formation of carcinogenic substance from dietary materials (Hunt, *et al*., 1980). It has been noted that patients with type 1 diabetes generally have low vitamin C levels. Therefore, by increasing the amount of vitamin C the blood stream, sorbitol a harmful sugar whose presence or rather accumulation results in increased risk of diabetes complications may be reduced.

The roles of vitamins D as an anti-ricket vitamin, E in sterility and K in blood clothing have long been noted. It has been reported that impaired pancreatic beta cell function, insulin resistance, and low-grade systemic inflammation are important risk factors of developing glucose intolerance and type 2 diabetes (Pittas, *et al*., 2004; Strick, *et al*., 2000). In both animal and human studies, impaired pancreatic beta cell function has been reported with vitamin D insufficiency (Baynes, *et al*., 1997; Chertow, *et al*., 1983; Inomata, *et al*., 1986; Normal, *et al*., 1980; Soliman, *et al*., 1987), while vitamin D supplementation restores insulin secretion (Borissova, *et al*., 2003; Inomata, *et al*., 1984; Orwoll, *et al*.,1994; Scragg, *et al*., 2004; Tanaka, *et al*., 1984). Studies in patients with type 1 diabetes have revealed an increased level of oxidative stress (Hannon-Fletcher, *et al*., 1999) and evidence has shown that such stress could be prevented when vitamin E is available (Jain, 1989). Hence, there lies above the possible benefits of having the appreciable quantity of these vitamins in the studied sample.

Result of mineral composition as presented in Table 3 clearly shows the presence of some micro minerals such as sodium (0.24±0.07%), potassium (0.13±0.01%), calcium (0.76±0.19%), magnesium (0.54±0.17%), and phosphorus (0.48±0.11 mg/100g); and micro-ones such as iron (0.09±0.01%), zinc (1.76±0.21 mg/100g), and copper (0.27±0.10 mg/100g) in the studied leaf sample.

Table 3: Result of Minerals Composition of *V. doniana* Leaf Sample.

Minerals	Composition
Sodium (%)	0.24±0.07
Potassium (%)	0.13±0.01
Calcium (%)	0.76±0.19
Magnesium (%)	0.54±0.17
Phosphorus (mg/100g)	0.48±0.11
Iron (mg/100g)	0.09±0.01
Zinc (mg/100g)	1.76±0.21
Copper (mg/100g)	0.27±0.10
[Calcium]/[Phosphorus]	1.58
[Calcium]/[Magnesium]	1.37
[Sodium/potassium]	1.84

The absorption of micronutrients as well as vitamins is completed by minerals (Agomuo, *et al*. 2012, Okaka and Okaka, 2005). Despite associating high level of sodium in the body to hypertension, its role as osmotic pressure fluid when in association with potassium, at moderate concentration cannot be overemphasized (Olusanya, 2008). Osmotic active sodium molecules are prevented from accumulating inside cells by the Na^+/K^+ pump. According to Tim (2015), potassium is recognized as a means to prevent hypertension and stroke. The variation in sodium and potassium constituents of *V. doniana* leaf sample is particularly important when the leaf is taken into the body.

The biological association of calcium, magnesium, and phosphorus has been linked with the formation of strong bones and teeth (Agomuo, *et al*., 2012; Olusanya, 2008). The role of iron in coordinating nervous action, oxidation of sugars and proteins, formation of hemoglobin has been noted. Zinc and potassium are associated with insulin and in helping diabetic patients. Copper contributes to iron absorption and its deficiency results in cardiovascular diseases and other acute problems (Olusanya,

2008). It is also a constituent of many metalo-enzymes and facilitates incorporation of iron into red blood cells (Amadi, *et al.,* 2013).

[Calcium]/ [Phosphorus] ratio of the present study is 1.8 and higher than its critical value 1.0. [Calcium]/[Magnesium] recorded 1.37, which is less than its critical value of 2.20. [Sodium/potassium] value as estimated is 1.84 and is higher than its critical value of less than 1.0. The values sodium, potassium, calcium, magnesium, phosphorus, iron and copper minerals in the present study are higher than the values reported by Vunchi, *et al*. (2011) on fruit pulp of *V. doniana*.

Phytochemicals are plants secondary metabolites, which are bioactive in nature (James, *et al*. 2013). Extensive studies and research have linked these constituents to protection in animals as well as humans. Table of qualitative and quantitative phytochemical constituents of *V. doniana* leaf sample (Table 4) shows the presence of tannin, hydrogen cyanide (HCN), soluble sugars, reducing sugar, non-reducing sugars, flavonoids, saponins, steroids, terpenoids, phenol, alkaloids, and glycosides. Qualitatively, tannins, soluble sugars, flavonoids, terpenoids, phenols, and alkaloids were in high concentrations. Reducing sugars, non-reducing sugars, and steroids were in moderate concentrations while hydrogen cyanide (HCN), saponins, and glycosides were in low concentrations.

Table 4: Qualitative and Quantitative Phytochemical Constituents of *V. doniana* Leaf Sample.

Phytochemicals	Qualitative	Quantitative
Tannins	+++	17.97±0.11 (mg/100g)
Hydrogen cyanide (HCN)	+	0.66±0.14 (mg/100g)
Soluble sugar	+++	22.16±0.18 (mg/100g)
Reducing sugar	++	8.57±0.38 (mg/100g)
Non-reducing sugar	++	11.87±0.38 (mg/100g)
Flavonoids	+++	32.16±0.24 (mg/100g)
Saponins	+	0.85±0.10 (mg/g)
Steroids	++	3.20±0.18 (mg/g)
Terpenoids	+++	49.24±0.32 (mg/100g)
Phenol	+++	38.48±0.21 (mg/100g)
Alkaloids	+++	34.78±0.29 (mg/100g)
Glycosides	+	0.84±0.07 (mg/100g)

The qualitative presence of tannins, alkaloids and saponins in the leaf sample is in line with the earlier study of Tadzabia, *et al*. (2013) on elemental and phytochemical screening of *Vitex doniana* leaves and stem bark from in Hong Local Government Area of Adamawa State, Nigeria. The presence of tannins, flavonoids and glycosides agree with the earlier work of James, *et al*. (2013) on determination of phytochemical constituents of the aqueous extracts of the leaves, stem bark and root bark of *Vitex doniana* and its effects on lipid profile of albino rats.

Quantitatively, observed values for tannins, hydrogen cyanide, soluble sugars, reducing sugar, non-reducing sugars, flavonoids, saponins, steroids, terpenoids, phenol, alkaloids, and glycosides are 17.97±0.11 mg/100g, 0.66±0.14 mg/100g, 22.16±0.18 mg/100g, 8.57±0.38 mg/100g, 11.87 mg/100g, 32.16±0.24, 0.85±0.10 mg/g, 3.20±0.18 mg/g, 49.24±0.32 mg/100g, 38.48±0.21 mg/100g, 34.78±0.20, and 0.84±0.07 mg/100g respectively. From the result, terpenoids was the highest in terms of observed values followed by phenol and alkaloids while hydrogen cyanide was the least.

Basu, *et al*. (2007) noted that all phytochemicals have potential heath effect under some circumstances. Tannic compound is known for its astringency and hastens healing of wound and inflamed mucous membrane (Okwu and Okwu, 2004). Tannins of *V. doniana* leaf sample in the present study is higher than the values earlier reported by James, *et al*. (2013) on leaves, stem bark and root bark of the *V. doniana.* HCN is known for its potent activity of influencing the respiratory chain.

Soluble sugars, reducing sugar and non-reducing sugars are digestible and may influence the blood glucose level easily in case of insulin eventuality in the body. High constituents of soluble sugars as well as the moderate concentration of reducing sugar of the leaf sample could mean high glycemic max following consumption the leaves. The foamy, bitter taste, antimicrobial, hemolytic effect on red blood cells and hypocholesterolemia property of saponins has been reported (Okwu and Okwu, 2004). The saponin constituents of *V. doniana* leaves as observed in the present study is higher than the earlier values reported by James, *et al*. (2013) on leaves, steam bark and root bark of the same plant.

Steroidal compounds have been used to reduce stress, reduce cholesterol levels, activate immune system, enhance memory and learning and to treat tumor cells in cancer cases

(Visweswari, *et al*., 2013). Terpenoids are small molecular products synthesized by plants and are probably the most widespread group of natural products. Terpenoids show significant pharmacological activities, such as antiviral, antibacterial, antimalarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities (Mahato and Sen, 1990).

Alkaloids are used for the treatment of tumors, nocturnal leg cramps caused by vascular spasms, and diarrhea. They possess anti-microbial activity and sedative effects. Many alkaloids are anesthetics and have calming effects on psychotic or hypertensive patients without inducing sleep. Alkaloids can also be used to treat psychiatric and palpitation (Visweswari, *et al*., 2013). Glycosides can suppress and soothe irritant dry coughs. They have a helpful sedative and relaxant effect on the heart and muscles when taken in small doses. They are significantly diuretic (Visweswari, *et al*., 2013).

The studied leaf sample showed high constituents of steroid, terpenoids, alkaloids, and glycosides. The presence of steroids, terpenoids and alkaloids as observed in this study is not in line with the earlier study of James, *et al*. (2013), who reported their absence in leaves, stem bark and rook bark of *V. doniana*. Glycosides of the present study is higher compared to those of leaves, stem bark and root bark of the same plant as reported by James, *et al*. (2013). Flavonoids and phenolic acids are polyphenols. Phenolic acid present in certain plants and fruit has proved to be effective against high fat induced hyperlipidemia and oxidative stress through regulation of insulin secretion, lipogenic enzymes and regulation of antioxidant (Lacueva, *et al*., 2011). The leaf sample of *V. doniana* recorded high values of flavonoids and phenols when compared to those of *G. kola* and *A. melegueta* (Okwu, 2005).

Table 5 shows the result of GC-MS analysis of crude methanolic extract of *V. doniana* leaves. The Table shows the presence of Di-n-octyl phthalate ($C_{24}H_{38}O_4$) with an areas of 35.84%, and a retention time of 24.87 min; ethyl ester palmitate ($C_{18}H_{36}O_2$) had an area of 15.54% with a retention time of 18.15 min. 2,3,5,8-tetramethyldecane ($C_{14}H_{30}$) had an area of 7.32% with a retention time of 19.91 min; methyl 14-methylpentadecanoate ($C_{17}H_{34}O_2$) with an area of 6.61% and a retention time of 16.78 min; palmitic acid had 5.46 ($C_{16}H_{32}O_2$) had an area of 5.48% with a retention time of 17.81 min; ethyl ester-9-octadecenoate ($C_{20}H_{38}O_2$) with area of 5.44% with a retention time of 20.83 min; 1-octadecene ($C_{18}H_{36}$) with an area of 4.81% and a retention time of 21.18 min; 3,4,5,6,-tetramethyloctane ($C_{12}H_{26}$) with an area of 4.55% and a retention time of 18.28 min; n-icosane ($C_{20}H_{42}$) with an area of 3.51% and a retention time of 22.42 min; n-dodecane with an area of 3.07% and a retention time of 21.25 min; 1-pentylheptyl acetate ($C_{14}H_{28}O_2$) with an area of 2.69% and a retention time of 23.42 min; methyl ester decanoic acid ($C_{11}H_{22}O_2$) with an area of 2.18% and a retention time of 20.28 min; and 2-([(1-Cyano-1methylethyl)amino]carbonyl)benzoic acid ($C_{12}H_{12}N_2O_3$) with an area of 1.63% and a retention time of 15.74%.

Di-n-Octylphthalate had the highest retention time of 24.87 min. and an area of 35.84%. 1-Pentylheptyl acetate with the retention time of 23.42 min, and an area of 2.69% was the next in terms of retention time. The importance of these constituents lies in their physiological effects in plants, animals, and humans as well as in industries.

Di-n-Octyl phthalate, Ethyl ester palmitate, n-Icosane, n-Dodecane for instance are of industrial importance. 2,3,5,8-Tetramethyldecane play a role in human metabolism, and it is among the metabolites of cancer metabolism. Palmitic acid is among the saturated fats found in animals and plants. It is also used as food additives and as surfactant in cosmetics.

Table 6 shows the membrane stabilization activity of crude methanolic extract of *V. doniana* leaf sample on rat erythrocyte membrane subjected to heat and hypotonic stresses. From the Table, membrane stabilization of the leaf extract was 66.24±6.24% against the standard 59.78±4.25 (indomethacin). The crude methanolic extract of *V. doniana* leaf provided a better inhibition on erythrocyte membrane subjected to heat and hypotonic stresses against the standard (Table 6).

The mode of action of the leaf extract and standard anti-inflammatory drugs could relate to binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the hemolysis of red blood cells.

Table 5: Results of GC-MS Analysis of Crude Methanolic Extract of *V. doniana* Leaf Sample.

S/n	Ret. Time (min)	Area (%)	Compound	Formular
1	24.87	35.84	Di-n-Octyl phthalate	$C_{24}H_{38}O_4$
2	18.15	15.54	Ethyl ester palmitate	$C_{18}H_{36}O_2$
3	19.91	7.32	2,3,5,8-Tetramethyldecane	$C_{14}H_{30}$
4	16.79	6.61	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$
5	17.81	5.48	Palmitic acid	$C_{16}H_{32}O_2$
6	20.83	5.44	Ethyl ester-9-Octadecenoate	$C_{20}H_{38}O_2$
7	21.18	4.81	1-Octadecene	$C_{18}H_{36}$
8	18.28	4.55	3,4,5,6,-Tetramethyloctane	$C_{12}H_{26}$
9	22.42	3.51	n-Icosane	$C_{20}H_{42}$
10	21.25	3.07	n-Dodecane	$C_{12}H_{26}$
11	23.42	2.69	1-Pentylheptyl acetate	$C_{14}H_{28}O_2$
12	20.28	2.18	Methyl ester decanoic acid	$C_{11}H_{22}O_2$
13	15.74	1.63	2-([(1-Cyano-1methylethyl)amino]carbonyl)benzoic acid	$C_{12}H_{12}N_2O_3$
14	16.28	1.35	3,4,5,6- Tetramethyloctane	$C_{12}H_{26}$

Compounds are arranged in decreasing order of their peak areas.

Table 6: Membrane Stabilization Activity of Crude Methanolic Extract of *V. doniana* Leaf on Rat Erythrocyte Membrane Subjected to Heat and Hypotonic Stresses.

Test Substance	Membrane Stabilization (%)
Leaf extract	66.24±6.24
Standard (Indomethacin)	59.78±4.25

It has been reported that certain saponins and flavonoids exerted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro,* while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (Oyedapo, *et al*., 2004; El-Shabrany, *et al*., 1997; Middleton, 1996; Pathak, *et al*., 1991; Sadique, *et al*., 1989; Hess and Milloning, 1972 Van-Cangeghen, 1972). The present observation could mean that methanolic extract of the leaf contain substances that protected the erythrocytes membranes effectively.

CONCLUSION

The bioactive profiling of *V. doniana* in the present study revealed proximate composition, vitamins, minerals, and phytochemical (qualitative and quantitative) status of the leaf sample. The leaf sample showed high concentrations of terpenoids, phenol, alkaloids, soluble sugars, and tannins.

The GC-MS analysis of leaf sample revealed some compounds of physiological and industrial importance. These constituents could be behind the activity of the methanol extract of the leaf sample as observed on membrane stabilization of erythrocyte membrane of rat subjected to heat and hypotonic stresses in this study. This study has shown the bioactive profiling of *V. doniana* leaf and its effect on rat erythrocyte membrane stabilization activity.

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