Mediterranean Journal of
Pharmacy & Pharmaceutical Sciences
www.medjpps.com

ISSN: 2789-1895 online
ISSN: 2958-3101 print

ORIGINAL RESEARCH article

Chemical characterizations and anti-sickling potential of methanol extract of *Justicia carnea* (flamingo plant)

Igbinumwen Osamwonyi [1], **Osaro Iyekowa** [2 *], **Mabel I. Ebengho** [1], **Mary O. Edema** [3], **Amowie P. Oviawe** [2], **Sunday M. Momoh** [4], **Lucky O. Eduwuirofo** [2], **Rukevwe Iyekekpolor** [2], **Oghomwen T. Michael** [2], **Ruth O. Oghomwen** [2], and **Amanda A. Umeodinka** [2]

[1] Department of Haematology, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria
[2] Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria
[3] Department of Chemistry, Federal University of Petroleum Resources, Warri, Delta State, Nigeria
[4] Department of Clinical Chemistry, Forensic Science (Toxicology), University of Benin Teaching Hospital, Benin City, Nigeria
[*] Author to whom correspondence should be addressed

Received: 08-08-2024, **Revised:** 07-09-2024, **Accepted:** 13-09-2024, **Published:** 30-09-2024

HOW TO CITE THIS

Osamwonyi et al. (2024) Chemical characterizations and anti-sickling potential of methanol extract of *Justicia carnea* (flamingo plant). Mediterr J Pharm Pharm Sci. 4 (3): 65-75. [Article number: 171]. https://doi.org/10.5281/zenodo.13759785

Keywords: *Justicia carnea*, sickle cell, phytochemicals, chromatography

Abstract: *Justicia carnea*, a plant rich in bioactive compounds, has traditionally been used as a haemoglobin booster by herbal practitioners in rural areas of Edo State of Nigeria. In this study, the methanol leaves extract of *Justicia carnea* was investigated for its antisickling potential in hemoglobin SS (HbSS) *in-vitro* by sodium metabisulphite assay and characterized by Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). Terpenoids, saponins, glycosides, phenolics, flavonoids and alkaloids were present except tannins. Photomicrograph examinations revealed a reduction of sickle cells with a percentage decrease from 37.50%, and 22.22% down to 3.85% with 100 mg/mL crude extract of *Justicia carnea* at three durations, respectively. Detected compounds from Gas Chromatography-Mass Spectrometry included hexadecanoic acid methyl ester (Retention time (R_t): 21.46, 05.99%) and 9,12-octadecadienoic acid, methyl ester (R_t: 23.567, 02.59%) while the major constituent was phytol (R_t: 23.741, 67.28%). While kaempferol (R_t: 17.23, 16.72%) was the major component from High-Performance Liquid Chromatography quantification among resveratrol (R_t: 3.7, 13.85%), gallic acid (R_t: 5.88, 3.55%), Justicinol (R_t: 11.85, 01.34%) and phytic acid (R_t 5.06, 01.43 %). These compounds have been cited as physiological agents that reduce inflammation, and oxidative stress and potentially prolong the lifespan of cells across species. The antisickling results from this study corroborate and support the traditional use of the plant in treating patients with sickle cell anemia.

Introduction

Sickle cell disease is mostly prevalent in sub-Saharan Africa with nearly 90.0% of the world's Sickle Cell Anemia (SCA) population living in Nigeria and the Democratic Republic of Congo and other places such as India [1]. In Africa, the highest prevalence of sickle-cell trait lies between latitudes 15° North and 20° South and can be as high as 45.0% of the population in some areas [2]. The disease is characterized by lifelong hemolytic anemia and a variety of painful vaso-occlusive events, which occurs in 70,000 to 80,000 Americans of African, Mediterranean, or Middle Eastern extraction [3]. Prevalence levels decrease to 1.0% and 2.0% in

ISSN: 2789-1895 online
ISSN: 2958-3101 print

North Africa and less than 1.0% in Southern Africa. In countries like the Republic of Congo, Cameroon, Gabon, Ghana, and Nigeria, the prevalence is 20.0% and 30.0% while in some parts of Uganda, it is as high as 45.0% [4]. About 50.0% of the 150,000 children born with SCA annually in Nigeria are likely to die before their 10th birthday and the geographical distribution of sickle cell trait corresponds with regions of highest density of malaria [5]. This is believed to be due to the S allele in the heterozygotes conferring resistance to *Plasmodium falciparum* which improves an individual's fitness in malarial endemic areas [5]. However, the inheritance of the mutation at both alleles of hemoglobin SS (HbSS) predisposes individuals to severe malaria and increased mortality, as well as the effect of SCA [6]. Research on flora species has expanded globally to study the potential of medicinal plants used in various traditional systems [7]. Production and cost advantages of pharmaceuticals made from plants can attract more capital investment in research and the development of new therapeutics, giving patients access to new drugs faster. The anti-sickling potentials of some medicinal plants have been studied and these include whole parts of *Petiveria alliacea, Canna indica* and *Pergularia daemia* [8], *Caja nuscajan* seeds, *Zanthoxyllumz anthoxyloides* (Fagara) root, *Carica papaya* unripe fruit and leaves; and *Parquetin anigrescens* [9]. Aqueous extracts of the reddish brown freshly fallen leaves of *Terminalia catappa* have also exhibited anti-sickling activity on sodium metabisulphite-induced sickling [8].

Justicia carnea (flamingo plant), family- *Acanthaceae*, is a perennial shrub with a characteristic pink to purple or orange flower color that blooms all summer. It grows 3-4 feet wide and height of 1.8 m. The flowering plant occurs in Brazil, the Benin Republic, the Democratic Republic of Congo, Nigeria, and other African countries. It is propagated by pushing 1-2 inches of the stem into the soil and kept until new leaves appear [10]. The plant is a major player in the traditional treatment and management of anemia in sickle cell disease and other ailments in major areas of Edo State of Nigeria. Phytochemicals studies reported in aqueous leaf extract of *J. carnea* included terpenoids, tannins, alkaloids, carbohydrates, flavonoids, saponins, phenols and glycosides. In contrast, phenols and flavonoids were found in high concentrations which could be responsible for the anticancer and antioxidant activities exhibited by the plant [11]. Vitamins such as A, B_1, B_{12}, B_6, B_9, B_2, C and E have also been detected in the aqueous extract of *Justicia carnea* while iron, magnesium, calcium, and zinc were also found [11]. Chemical compounds isolated from *Justicia species* include steroids (campesterol, stigmasterol, sitosterol, and sitosterol-D-glucoside [12]; apigenin, justicidin, justiciocide and justicinol among others [13]. Several researchers have investigated its usefulness in ethno-medicine [7]. It is used to treat various health issues like diarrhea, typhoid, hepatitis, liver diseases, cancer, anemia, respiratory infections, inflammation, and sickle cell disease [14]. It's used as a blood booster, which is especially beneficial for people with anemia, menstruation pregnancy. People often grow it around their homes not only for its medicinal value but also for its aesthetic value [15]. Recent research utilizing animal models has unveiled the blood-boosting properties of *Justicia carnea*, which surpass those of conventional blood tonics [14]. Therefore, this study aims to characterize and investigate the anti-sickling potential of the methanol extract of *Justicia carnea* leaves.

Plate 1: Leaves of *Justicia carnea*

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Materials and methods

Collection of plant sample: Fresh leaves of the *Justicia carnea* were obtained from its natural habitats at Ogida Quarters in Egor local Government area of Edo State, Nigeria (in the morning during the dry season of February 20th, 2024). The leaves were identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The plants were dried for 28 days and pulverized into powder using a mechanical grinding machine in the laboratory at room temperature of 27.0°C.

The extraction procedure: 623 g of the pulverized plant was macerated in one liter of methanol solvent for 72 hrs. The mixture was sieved using No. 1 Whatman filter paper number 4. The filtrate was concentrated using a rotary evaporator and the crude extract was stored in the refrigerator below 4.0°C until used for further experiment.

Phytochemical screening of the extract: The screening was carried out on the *Justicia carnea* extracts using standard procedures [16]. The phytoconstituents investigated were glycosides, saponins, flavonoids, phenolics, eugenols, tannins, terpenoids, steroids and alkaloids.

Collection of blood samples: 5.0 mL of blood sample was obtained by vein puncture from one confirmed sickle cell patient (HbSS) not in crisis from the University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City, Nigeria. The erythrocytes were isolated from whole blood by centrifuging at 1,500 x g for 15 min. The plasma being siphoned out carefully from the sickle cell sediment was done by Pasteur pipette. Caution was observed to note that the patient had not been transfused for twelve months before the anti-sickling analysis, with Hb AA blood. All anti-sickling experiments were carried out with freshly collected blood. Only one type of blood sample was used at a time for the plant extract doses. To confirm the patient's sickle cell nature, the HbSS blood samples were first characterized by hemoglobin electrophoresis on cellulose acetate gel [17]. The sample was found to be sickle cell blood and was then stored at ±4.0°C in a refrigerator. Only blood samples presenting a good sickling rate (≥90.0%) were selected for anti-sickling activity.

Anti-sickling activity evaluation, sodium metabisulphite (SMBS) test: The antisickling activity was done using a modified method [18]. Vein-punctured blood samples from sickle cell anemia patients not in crisis were collected into EDTA bottles and centrifuged to remove the serum. The resulting packed erythrocytes were washed three times with 1.0 mL sterile normal saline per mL of blood. This study was approved by the Ethical Committee on the use of specimens/animals, University of Benin, Benin City, Edo State, Nigeria (2024). The samples were then centrifuged each time for five min at a speed of 2,000 rpm to remove the supernatant, and 0.5 mL of the washed erythrocytes were mixed with 0.5 mL of the different extract doses in uncovered test tubes. Samples were taken from the different mixtures and incubated at 37.0°C for 3.0 hrs. while shaking occasionally. 0.2 ml of 2.0% SMBS was added to deoxygenate the system, mixed thoroughly and sealed with liquid paraffin samples before incubating at 37.0°C, and samples were taken at 45 min intervals until three readings were obtained. The sample of plant extract (100 mg/mL) was smeared on a microscope slide, fixed with 95.0% methanol, dried, and stained with Leischman's stain. The sample was examined under the oil. An immersion light microscope was used to count the red blood cells in each sample from five different fields of view across the slide. The numbers of sickle and unsickle red blood cells were noted. Negative control was achieved by SMBS (5.0 mg/mL) as a reductant or deoxygenating agent [19].

Calculation of sickled red blood cell

$$\text{Percentage sickled red blood cell} = \frac{\text{Sickle cells}}{\text{RBC count}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

5 mg per mL = 500 mg per L and RBC = Red blood cell

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Isolation of plant extracts

Vacuum liquid chromatographic (VLC) analysis: The crude methanol extract was combined uniformly with silica gel (1: 1) and subjected to VLC using silica gel (60-120 mesh) as the stationary phase. The sample was eluted with the aid of a vacuum pump using a combination of solvents consisting of 100% hexane, hexane: ethyl acetate (1: 1), and ethyl acetate (100%) solvents as mobile phase. Each of the three eluted fractions was respectively concentrated and monitored using thin-layer chromatography (TLC). The three fractions eluted from the VLC were examined by preparatory TLC plates in a few suitable solvent systems to obtain the retention factor (R_f) of any components that appeared as spots. Fractions with similar R_f values were combined, dried and coded. Isolated brown oil-coded sample A (R_f 0.75, solvent system: hexane: acetate 7: 3) was obtained from 100% hexane crude fraction while sample C (light brown solid, R_f 0.65, solvent system: 100% ethyl acetate) was recovered from 100% ethyl acetate crude fraction. Sample A (oil) was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis while sample C (solid) was characterized by High Performance Liquid Chromatography (HPLC) analysis. The second eluted fraction of hexane: ethyl acetate (1: 1) from VLC was not characterized due to the strayed nature of the TLC appearance.

GC-MS analysis: The GC-MS analysis of the isolated oil (sample A) of the methanol extract of *Justicia carnea* was done on a Shimadzu, GCMS-QP2010SE. Separation of the oil was carried out on a HP-5 MS (5.0% phenylsiloxane) column with nitrogen as the carrier gas with a flow rate, of 1.80 ml/min. The oven programme was set at a temperature of 70.0^{o}C and held for two min, then it was ramped at a rate of 10.0^{o}C per min to 280^{o}C and held for seven min. The oil sample was introduced via an all-glass injector working in the split mode, with a helium carrier gas low rate of 1.2 ml per minute. The identification of the oil chemical constituents was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS. The retention indices, peak area percentage and mass spectra fragmentation pattern of the chromatogram of the oil samples were compared with the database of the National Institute of Standards and Technology (NIST), NIST08.LIB [20] for possible identification of the name, molecular weight, formula, and structure of the chemical constituents in the oil sample.

HPLC analysis

Preparation of standard: 1.2 mg each of phenolic, flavonoid, and anthocyanins standards were taken in 0.1% phosphoric acid in water (HPLC grade). From which 20.0 µL were injected into the HPLC system for making a standard curve.

Preparation of plant sample: 10.0 g of solid sample from ethyl acetate crude fraction were extracted with acetonitrile and the extract stabilized with ethyl acetate in a 25.0 mL standard flask and made up to the mark.

HPLC Procedure: The constituents of the solid sample were characterized by HPLC analysis using the method previously described [20]. The solid sample and the three standards (phenolics, flavonoid, and anthocyanins) were subjected to HPLC using a 600 series HPLC pump and 2487 dual wavelength UV detector-254 and 360 nm of bioazyemes, Bangalore having reprobond C18 column-4.6x250 mm and 7725 Rheodyne injectors. The HPLC instrument was operated at room temperature (23.0±2.0°C). 10.0 g of each of the solid samples was extracted with acetonitrile, and the extracts stabilized with ethyl acetate in a 25.0 mL standard flask. 5.0 µL of extracts were respectively injected into the HPLC at a flow rate of 02.0 mL/min and the peak area was reported and used for quantification. The compounds eluted with two solvents such as acetonitrile and 0.1% phosphoric acid in water were used for the detection of the external standards. The total run time of the program was 20 min.

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Results

This study indicated that terpenoids, saponins, glycosides, phenolics, flavonoids and alkaloids except tannins are found as shown in **Table 1**. **Plates: A, B, C, D, and E** showed the photomicrographs of the sickle cell blood examined upon investigation with negative control (SMBS) (**Plate: A**) and methanol extract of *Justicia carnea* (**Plates: B, C, D, and E**). The time duration of exposures to the plant extracts, number of sickle cells, number of unsickled cells and appearance of the red blood cells are presented in **Table 2**. The GC-MS chromatogram of the isolated brown oil given in **Figure 1** showed ten peaks indicating from the search list of the chemical abstract service ten compounds. The chemical compounds identified (according to the NIST Library) in the oil fraction are presented in **Table 3**. The HPLC chromatogram of the solid sample coded sample C from methanol extract of *Justicia carnea* is indicated in **Figure 3** while the chemical constituents are shown in **Table 4**.

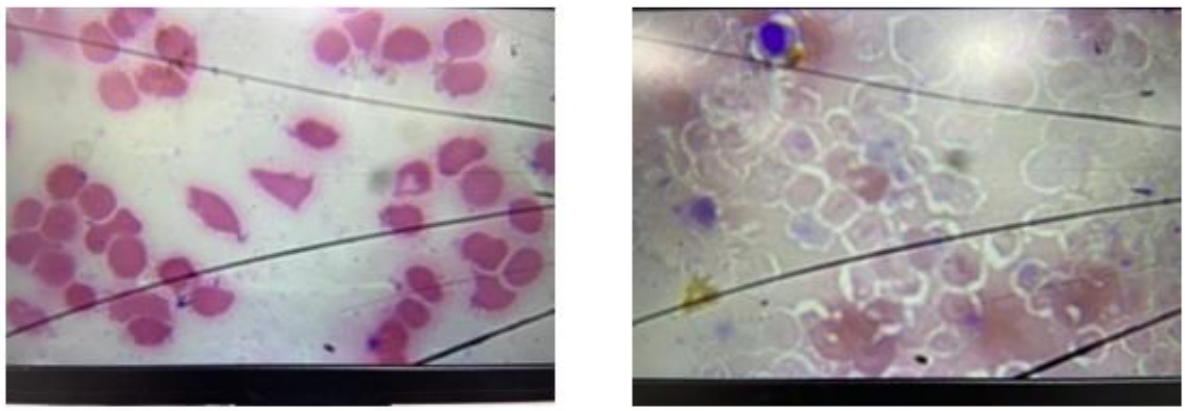
Table 1: Phytochemical screening of methanol extract of *Justicia carnea*

S/N	Phytochemical constituents	Methanol extract of *Justicia carnea*
1	Glycoside	+
2	Saponin	+
3	Flavonoid	+
4	Phenolics	+
5	Tannin	-
6	Eugenol	+
7	Steroid	+
8	Terpenoids	+
9	Alkaloids	+

+ = present - = absent

Table 2: Antisickling activity of methanol extract on HbSS blood

Duration of exposure of extract (min)	Number of sickles cell (%)	Number of non-sickle cell (%)	Appearance of cell
00	15 (37.50)	25 (62.50)	Moderate, hypochromic and macrocytic cells.
45	04 (22.22)	14 (77.78)	Few ovalocytes, macrocytic red cells
90	01 (3.85)	25 (96.15)	Macrocytic red cells
135	05 (12.82)	34 (87.18)	Ovalocytes, poikilocytes.



A B

Plate A: Optical microphotography of untreated HbSS blood erythrocytes (control), NaCl 0.9%, 2% $Na_2S_2O_5$, (Magnification x 100). **Plate B:** Optical Micrograph of 100mg/mL of methanol extract of *Justicia carnea* on sickle cell blood at 0 minutes (Magnification x 100)

ISSN: 2789-1895 online
ISSN: 2958-3101 print

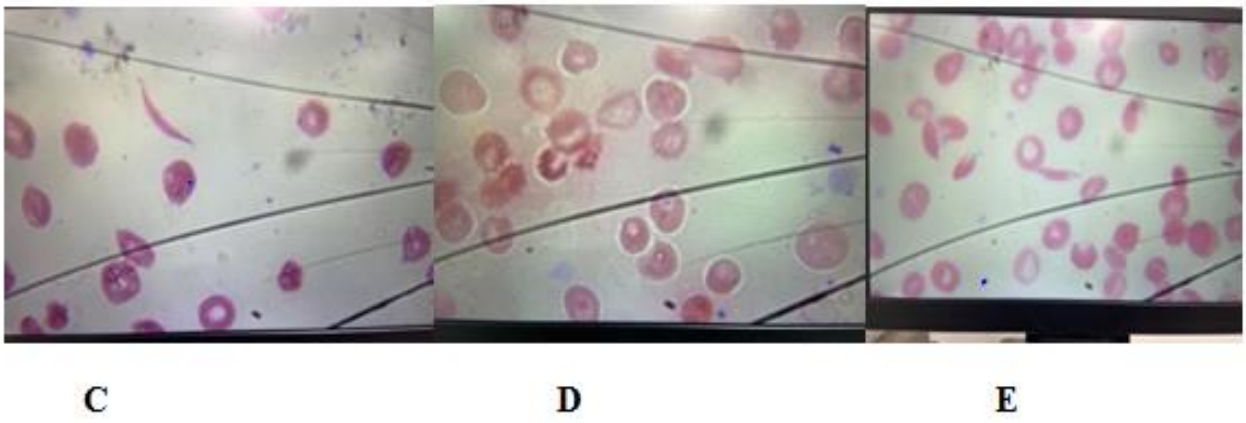
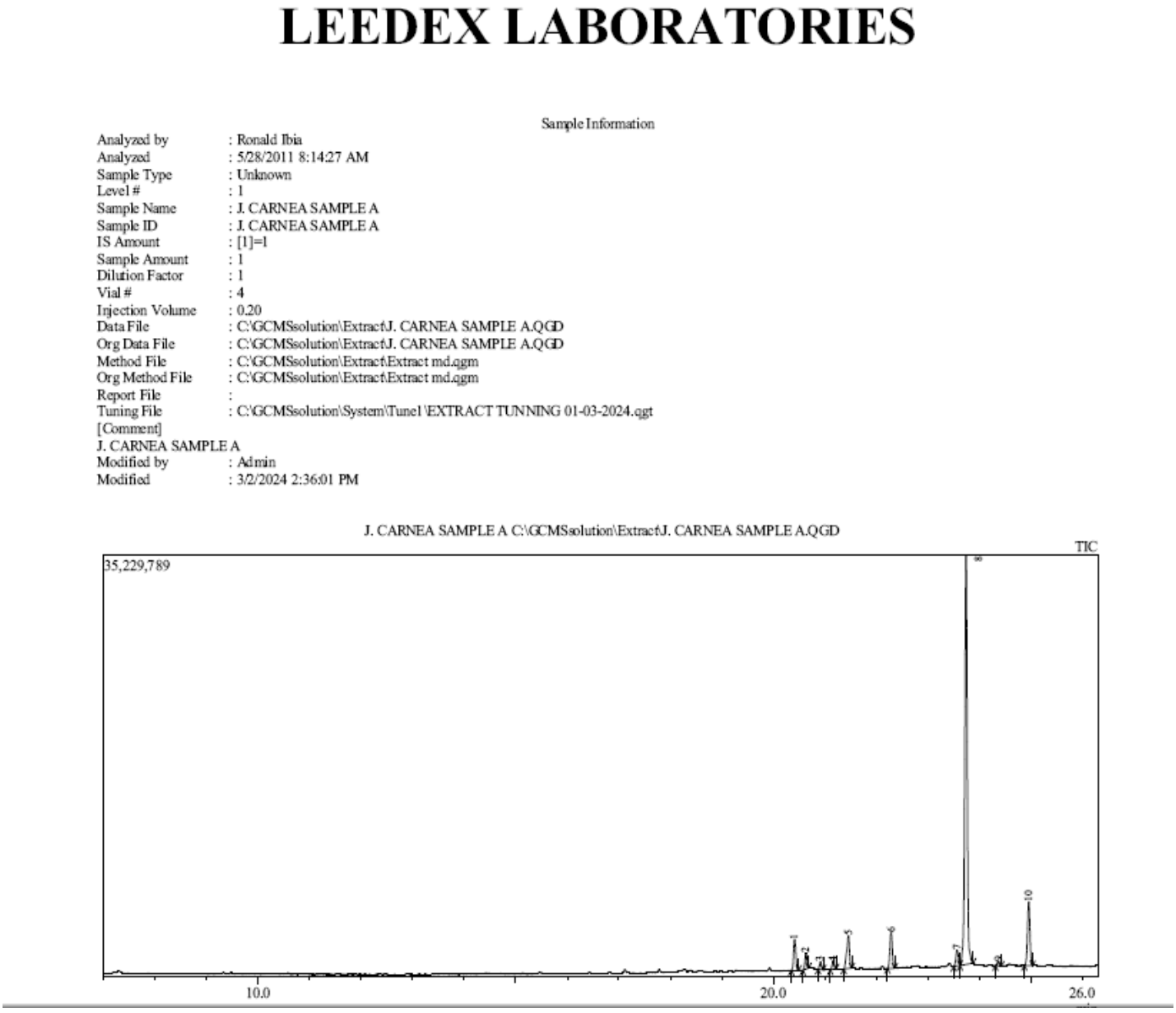


Plate C: Optical micrograph of 100 mg/mL of methanol extract of *Justicia carnea* on sickle cell blood at 45 min (Magnification x 100). **Plate D**: Optical Micrograph of 100 mg/mL of methanol extract of *Justicia carnea* on sickle cell blood at 90 min (Magnification x 100). **Plate E:** Optical Micrograph of 100 mg/mL of methanol extract of *Justicia carnea* on sickle cell blood at 135 min (Magnification x 100)



LEEDEX LABORATORIES

Sample Information

Analyzed by	: Ronald Ibia
Analyzed	: 5/28/2011 8:14:27 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: J. CARNEA SAMPLE A
Sample ID	: J. CARNEA SAMPLE A
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 4
Injection Volume	: 0.20
Data File	: C:\GCMSsolution\Extract\J. CARNEA SAMPLE A.QGD
Org Data File	: C:\GCMSsolution\Extract\J. CARNEA SAMPLE A.QGD
Method File	: C:\GCMSsolution\Extract\Extract md.qgm
Org Method File	: C:\GCMSsolution\Extract\Extract md.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tunel \EXTRACT TUNNING 01-03-2024.qgt
[Comment]	
J. CARNEA SAMPLE A	
Modified by	: Admin
Modified	: 3/2/2024 2:36:01 PM

J. CARNEA SAMPLE A C:\GCMSsolution\Extract\J. CARNEA SAMPLE A.QGD

TIC

35,229,789

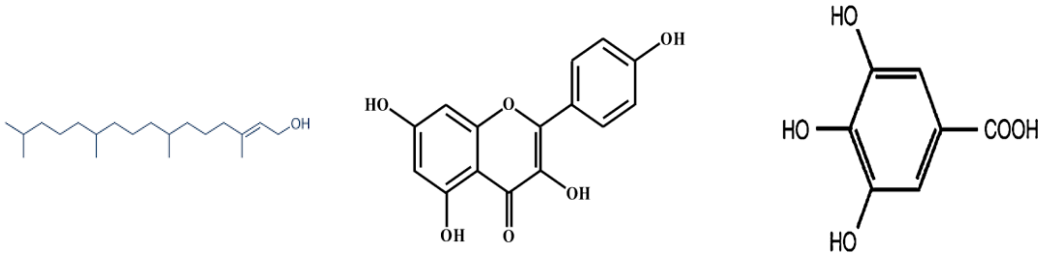
10.0 20.0 26.0

Figure 1: GC-MS analysis of isolated brown oil of methanol extract of *Justicia carnea*

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Table 3: Compounds in oil fraction of methanol extract of *Justicia carnea*

Peak number	Retention time (R_t)	Name of compounds	Area percentage
1	20.416	Tetradecyl-Oxirane	4.08
2	20.633	6,10,14-trimethyl-2-Pentadecanone	2.02
3	20.915	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.96
4	21.164	1,2-Benzenedicarboxylic acid	1.03
5	21.460	Hexadecanoic acid, methyl ester	5.99
6	22.288	Dibutyl phthalate	5.35
7	23.567	9,12-octadecadienoic acid, methyl ester	2.59
8	23.741	Phytol	67.28
9	24.364	Palmitoyl chloride	0.61
10	24.958	Phytol, acetate	10.10
Total		**100.00**	



(I) (II) (III)

Figure 2: Chemical constituents detected in *Justicia carnea* isolates: (I) Phytol (II) Kaempferol (III) Gallic acid

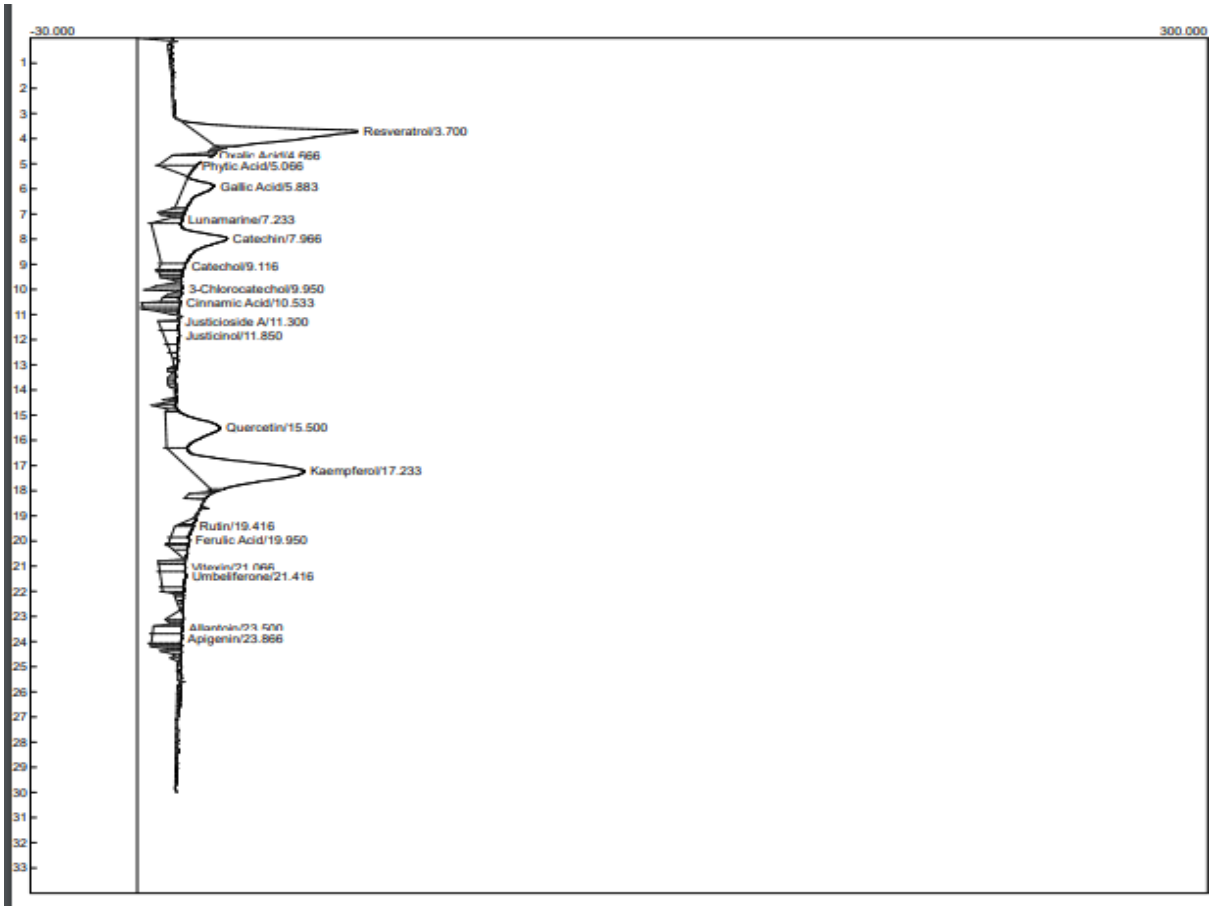


Figure 3: HPLC profile of fraction C of methanol extract of *Justicia carnea*

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Table 4: HPLC profile of ethyl acetate extract of *Justicia carnea*

Peak number	Retention time (R_t) (mins)	Name of compound	Peak area
1	3.700	Resveratrol	1385.0720
2	4.666	Oxalic acid	266.9140
3	5.066	Phytic acid	143.4755
4	5.883	Gallic acid	355.8700
5	7.233	Lunamarine	71.8460
6	7.966	Catechin	1161.6080
7	9.116	Catechol	105.2300
8	9.950	3-Chlorocatechol	78.5665
9	10.533	Cinnamic acid	105.0630
10	11.300	Justicioside A	109.3360
11	11.850	Justicinol	134.4030
12	15.500	Quercetin	860.0830
13	17.233	Kaempferol	1672.4940
14	19.416	Rutin	131.6210
15	19.950	Ferulic acid	81.0800
16	21.066	Vitexin	130.5020
17	21.416	Umbeliferone	246.5895
18	23.500	Allantoin	147.6610
19	23.866	Apigenin	190.4295

Discussion

The alkaloids in *Justicia* species are valuable in traditional pharmaceutical applications due to their broad spectrum of pharmacological activities [22]. The presence of glycosides aligns with the results reported by Bedoya and others [23] while glycosides were also reported [24]. The presence of phenolics, flavonoids and saponins in the extracts of *Justicia carnea* was identified as strong antioxidants and anti-inflammatory agents with the potential to protect the heart for optimal function [7, 13]. The report of Peter and others [25] has slightly been different with the presence of tannins in quantitative investigation using methanol as a solvent. In the current study, **plate** A shows an optical micrograph of the sickle cell blood alone (control group) while other plates are the photomicrographs of sickle cell blood in the presence of 100 mg/mL methanol extracts of *Justicia carnea* at different durations of exposures in minutes. **Plate B** showed moderate hypochromic and macrocytic red blood cells at the initial start of the experiment (zero min). However, as time progresses from zero min (**plate B**), 45 min (**plate C**) to 90 min (**plate D**). There was an observed reduction in the number of sickle cells with a decrease from 37.50%, and 22.22% down to 3.85%, respectively. Macrocytic red blood cells were pronounced in all the fields examined but there was no sign of sickling, an indication that the plant extract can sustain anti-sickling of the sickle cells. However, at the last duration of the experiment (135 min), there were observed ovalocytes and poiklocytes of the red blood cell but without sickling. In comparison with another species of *Justicia* (*Justicia seconda*), the plant extract exhibited anti-sickling activity with the anthocyanin's components quantified phytochemically. The reversal of sickle cell was sustained throughout the experiment [17]. Apart from anthocyanins, they also reported that medicinal plants with phenolics and terpenoid compounds show anti-sickling activities and, in this research, the phytochemical investigation also indicated both components. Other medicinal plants recently studied with antisickling potentials included *Pergularia daemia* and *Canna indica* which indicated a reversal of sickle cell to normal doughnut shape at 90 min of examination [8].

The main constituents detected from the isolated brown oil of *Justicia carnea* obtained from the GC-MS analysis of methanol fraction revealed important physiological chemical agents with nutritional and medicinal values. Detected compounds include hexadecanoic acid methyl ester (R_t: 21.46, 05.99%) and 9,12-octadecadienoic acid, methylester (R_t: 23.567, 02.59%) while the major constituent was phytol (R_t: 23.741,

ISSN: 2789-1895 online
ISSN: 2958-3101 print

67.28%). Hexadecanoic acid of 10.5% has been reported in the ethanol leaves extract of *Justicia carnea* [26]. It has shown antimicrobial activity, anti-inflammatory potency and other metabolic functions [27]. Phytol was observed to be the most abundant component in the oil fraction. It is a diterpenoid that possesses relevance in the synthesis of vitamin E and has been reported to modulate the transcription in plant and animal cells. This supports the previous research which opined that terpenoids and phenolics possess anti-sickling activity [17].

The HPLC profile data of the sample solid fraction of methanol extract of *Justicia carnea* revealed the highest peak to be kaempferol (R_t: 17.23, 16.72%). 19 compounds were identified from the analysis which includes resveratrol (R_t: 3.7; 13.85%), gallic acid (R_t: 5.88, 03.55%), and justicinol (R_t: 11.85, 01.34%). More so, phytic acid (R_t: 5.06, 01.43 %) has also been detected as a derivative of phytol earlier detected by GC-MS in this study. The presence of justicinol aligns with the previous findings [27]. In addition, allantoin with anti-inflammatory effect has been reported for *Justicia spicigera* but not for *Justicia carnea* as observed in the current study while justicioside A, kaempferol and Justicinol have all been isolated from *Justicia betonica* [7, 28]. Gallic acid is a vital metabolite encountered across diverse plant species. Its pharmacological attributes encompass antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [29]. Resveratrol, a polyphenol, reduces inflammation and oxidative stress and potentially prolongs the lifespan of cells across species [30]. While kaempferol, a flavonoid, is antimicrobial, anti-inflammatory, regulator of macrophages, and reduces the blood glucose level [31, 32].

Conclusion: The anti-sickling data of the methanol extract of *Justicia carnea* corroborates the traditional use of the plant in the treatment of sickle cell anemia. Thus, *Justicia carnea* contains important phytochemicals and bioactive components like resveratrol, kaempferol, phytol, and gallic acid that possess medicinal benefits.

References

1. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, Smith WR, Panepinto JA, Weatherall DJ, Costa FF, Vichinsky EP (2018) Sickle cell disease. Nature Reviews, Disease Primers. 4 (1): 18010, 1-22. doi: 10.1038/nrdp.2018.10
2. Olowoyo P, Akinyemi R, Owolabi M, Fawale B, Ogunniyi A (2017) Sickle cell trait appears to increase the risk of hemorrhagic stroke in African adults with dyslipidaemia: A pilot study. Austin Journal of Cerebrovascular Disease and Stroke. 4 (4): 1067. doi: 10.26420/austinjcerebrovascdisstroke.2017.1067
3. Steinberg MH (1999) Management of sickle cell disease. The New England Journal of Medicine. 340 (13): 1021-1030. doi: 10.1056/NEJM199904013401307
4. Cyril-Olutayo MC, Agbedahunsi JM, Akinola NO (2018) Studies on the effect of a nutritious vegetable, Telfairia occidentalis, on HbSS blood. Journal of Traditional and Complementary Medicine. 9 (2): 156-162. doi: 10.1016/j.jtcme.2017.08.013
5. Uyoga S, Macharia AW, Ndila CM, Nyutu G, Shebe M, Awuondo KO, Mturi N, Peshu N, Tsofa B, Scott JAG, Maitland K, Williams TN (2019) The indirect health effects of malaria estimated from health advantages of the sickle cell trait. Nature Communications. 10 (1): 856. 1-7. doi: 10.1038/s41467-019-08775-0
6. Friedman MT, West KA, Bizargity P, Annen K, Jhang JS (2018) The perils of transfusing the sickle cell patient. In: Immunohematology and Transfusion Medicine. 135-141. Springer International Publishing, US. 2nd ed., ISBN: 9783319909608. doi: 10.1007/978-3-319-90960-8
7. Sami A, Usama M, Saeed MM, Akram M (2021) Medicinal plants with non-steroidal anti-inflammatory-like activity. Mediterranean Journal of Pharmacy and Pharmaceutical Sciences. 1 (3): 25-32. doi: 10.5281/zenodo.5534605
8. Iyekowa O, Okieimen FE, Ehisuoria CO (2023) In-vitro antisickling activity of *Pergulariadaemia*, *Cannaindica* and *Petiveria alliacea* plants used in the treatment of sickle cell anaemia in Edo State, Nigeria. Tanzania Journal of Science. 49 (2): 433-445. doi: 10.4314/tjs.v49i2.14
9. Imaga NOA (2010) The use of phytomedicines as effective therapeutic agents in sickle cell anemia. Scientific Research and Essays. 5 (24): 3803-3807. doi: 10.5897/SRE.9000002

ISSN: 2789-1895 online
ISSN: 2958-3101 print

10. Mabberley DJ (1997) The plant book: a portable dictionary of the vascular plants. Cambridge University Press. 9: 1-9. ISBN: 0521414210.
11. Orjiakor CA, Uroko RI, Njoku OU, Ezeanyika LUS (2019) Nutritive properties of aqueous extract Justicia carnea leaves and its effects on haematological and some biochemical indices of anaemia induced male Wistar albino rats. Biomedical Research. 30 (4): 645-654. doi: 10.35841/biomedicalresearch.30-18-666
12. Rajakumar N, Shivanna MB (2009) Ethno-medicinal application of plants in the eastern region of Shimoga district, Karnataka India. Journal of Ethnopharmacology. 126 (1): 64-73. doi: 10.1016/j.jep.2009.08.010
13. Corrêa GM, de C. Alcântara AF (2012) Chemical constituents and biological activities of species of Justicia - a review. Revista Brasileira de Farmacognosia. 22 (1): 220-238. doi: 10.1590/S0102-695X2011000196
14. Wood J, Yasmin-Karim S, Moreau S, Kumar R, Akwanwi J, Derek A, Atoneche F, Kress J, Ngwa W (2020). Characterization of isolated extracts from justicia plant leaves used as remedy for anemia. Molecules. 25 (3): 534. doi: 10.3390/molecules25030534
15. Badami S, Aneesh R, Sankar S, Sathishkumar MN, Suresh B, Rajan S (2003) Antifertility activity of *Derris brevipes* variety coriacea. Journal of Ethnopharmacology. 84 (1): 99-104. doi: 10.1016/s0378-8741(02)00288-x
16. Sofowora A (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books limited Ibadan. 289.
17. Mpiana PT, Ngbolua KN, Bokota MT, Kasonga TK, Atibu EK, Tshibangu DS, Mudogo V (2010) In vitro effects of anthocyanin extracts from Justicia secunda Vahl on the solubility of haemoglobin S and membrane stability of sickle erythrocytes. Blood Transfusion. 8 (4): 248-254. doi: 10.2450/2009.0120-09
18. Sofowora EA, Issac WA (1979) Reversal of sickling and crenation in erythrocytes by the root of Fagara zanthoxyloides. LIoydia. 34 (4): 383-385. PMID: 4950991.
19. Iwu MN, Igboko AO, Onwubiko H, Ndu UE (1988) Effect of *cajanuscajan* from *Cajanus cajan* on gelation and oxygen affinity of sickle cell hemoglobin. Journal of Ethnopharmacology. 22: 99-104. doi: 10.1016/0378-8741(88)90118-3
20. Stein SE (2011) National Institute of Standards and Technology (NIST), Mass Spectral Database and Software, Version 3.02, USA. 32, 1-65.
21. Drust RW, Wrolstad RE (2001) Separation and characterization of Anthocyanins by HPLC. Current Protocols in Foods Analytical Chemistry. 1-13. doi: 1001002/0471142913.FAF0103S00
22. Kittakoop P, Mahidol C, Ruchirawat S (2014) Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. Current Topics in Medicinal Chemistry. 14 (2): 239-252. doi: 10.2174/1568026613666131216105049
23. Bedoya LM, Alvarez A, Bermej M, Gonzalez N, Beltran M, Sanchez Palomino S, Cruz SM, Gaitán I, Del-Olmo E, Escarcena R, Garcia PA, Cáceres A, Feliciano AS, Alcamí J (2008) Guatemalan plants extract as virucides against HIV-1 infection. Phytomedicine. 15 (6-7): 520-524. doi: 10.1016/j.phymed.2007.10.006
24. Fu G, Pang H, Wong YH (2008) Naturally occurring phenylethanoid glycosides: potential leads for new therapeutics. Current Medicinal Chemistry. 15 (25): 2592-2613. doi: 10.2174/092986708785908996
25. Peters DE, Ahaotu O, Wegwu M (2022) HPLC, GC quantitative characterization of phytochemicals In Justicia Carnea Leaf. International Journal of Novel Research in Life Sciences. 9 (5): 1-9. doi: 10.5281/zenodo. 7037574
26. Otuokere IE, Amaku AJ, Igwe KK, Chinedum GC (2016) Medicinal studies on the phytochemical constituents of Justicia carnea by GC-MS Analysis. American Journal of Food Science and Health. 2 (4): 71-77. doi: Nil.
27. Ugboko HU, Nwinyi O, Oranusi SU, Fatoki TH, Omonhinmin CA (2020) Antimicrobial importance of medicinal plants in Nigeria. The Scientific World Journal. 2020: 7059323. doi: 10.1155/2020/7059323
28. Niu CS, Chen W, Wu HT, Cheng KC, Wen YJ, Lin KC, Cheng JT (2010) Decrease of plasma glucose by allantoin, an active principle of yam (Dioscorea spp.), in streptozotocin induced diabetic rats. Journal of Agricultural and Food Chemistry. 58 (22): 12031-12035. doi: 10.1021/jf103234d
29. Dias MC, Pinto D, Silva AMS (2021) Plant flavonoids: chemical characteristics and biological activity. Molecules. 26 (17): 5377. doi: 10.3390/molecules26175377
30. Zhang L, Li C, Kakar MU, Khan MS, Wu P, Amir RM, Dai D, Naveed M, Li Q, Saeed M, Shen J, Rajput SA, Li J (2021) Resveratrol (RV): A pharmacological review and call for further research. Biomedicine and Pharmacotherapy. 143: 112164. doi: 10.1016/j.biopha.2021.112164
31. Fang SH, Rao YK, Tzeng YM (2005) Inhibitory effects of flavonol glycosides from *Cinnamomum osmophloeum* on inflammatory mediators in LPS/IFN-gamma activated murine macrophages. Bioorganic and Medicinal Chemistry. 13 (7): 2381-2388. doi: 10.1016/j.bmc.2005.01.050
32. Periferakis A, Periferakis K, Bădărău IA, Petran M, Popa D, Căruntu A, Costache R, Scheau C, Căruntu C, Costache DO (2022) Kaempferol: antimicrobial properties, sources, clinical, and traditional applications. International Journal of Molecular Sciences. 23 (23): 15054. doi: 10.3390/ijms232315054

ISSN: 2789-1895 online
ISSN: 2958-3101 print

Acknowledgments: The authors wish to appreciate the grant given for this research work from IBR, 2023 of the University of Benin, Benin City, Nigeria and funded by the Tertiary Education Trust Fund (TETFUND), Nigeria.
Authors' contributions: IO, OI, MIE & SMM conceived and designed the study. All authors participated in data collection IO, OI, MIE, MOE, APO & SMM performed the data analysis and interpretation of data. OI drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author's declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.