Available online at https://ifejost.oauife.edu.ng

IFE JOURNAL OF SCIENCE AND TECHNOLOGY

Vol 7. No. 1 (2023) 1–11

In vitro anti-inflammatory evaluation and GC-MS identified constituents of *Hippeastrum amaryllis* flower extract

A. Godwin[1], B. A. George[2], B. A. Akinpelu[3] and M. A. Aderogba[2*]
[1]Department of Biochemistry, Adeleke University, Ede, Osun State, Nigeria.
[2]Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.
[3]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria.

*Corresponding author: marogba@oauife.edu.ng
Tel: +234 803 581 5107

Abstract

This study investigated the erythrocyte membrane stabilization and anti-denaturant activities of *Hippeastrum amaryllis* flower extract and fractions; and profiled the constituents of the n-hexane active fraction using gas chromatography-mass spectrometry (GC-MS) hyphenated techniques. These were with the view to determining the anti-inflammatory potential of the flower extract and identifying its therapeutic constituent(s) for management of inflammatory conditions. The *H. amaryllis* pulverized flower sample was extracted with 80% (v/v) methanol for 72 hrs and filtered. The filtrate was concentrated *in vacuo* to obtain the crude methanol extract. The crude extract was suspended in distilled water and partitioned in turn using *n*-hexane and dichloromethane (DCM) to obtain two solvent fractions. These were separately concentrated *in vacuo* to produce *n*-hexane and DCM fractions, respectively. Erythrocyte membrane stabilization and anti-denaturant assays were performed on the crude and fractions. The *n*-hexane fraction that showed the highest anti-inflammatory potency was profiled using GC-MS method. Results showed that the percentage erythrocyte membrane stabilizing activity of *n*-hexane fraction (81.26 ± 0.00%) was significantly higher than the DCM fraction (20.14 ± 0.001%) and crude extract (15.57 ± 0.001%); and competed favourably with the standard agent, diclofenac sodium (84.78 ± 0.003%). Also, the *n*-hexane fraction elicited significant anti-denaturant activity (93.62 ± 0.01%) than DCM fraction (72.91 ±0.001%) and diclofenac sodium (86.20 ± 0.00%). The GC-MS result of the *n*-hexane fraction identified fifteen (15) compounds in which oleic acid (16.39%), campesterol (14.34%), *n*-hexadecanoic acid (13.68%) and 11-octadecanoic acid (9.32%) were the major constituents. These compounds have been reported as anti-inflammatory compounds which supported the finding of the study. The study concluded that *H. amaryllis* flower extract had anti-inflammatory property which could be attributed to oleic acid, campesterol and n-hexadecanoic acid.

***Keywords*:** *Hippeastrum amaryllis*; anti-inflammatory; membrane stability; tissue protein denaturation

Introduction

Hippeastrum amaryllis is an important member of Amaryllidaceae native to South America (Cimmino *et al*., 2017). Members of *Hippeastrum* genera are known to contain bioactive

metabolites phenanthridine alkaloids (especially lycorine). Earlier studies on Amaryllidaceae showed that lycorine was the predominant alkaloid responsible for several biological activities (Nair *et al*., 2017; Cimmino *et al*., 2017). Since the isolation of lycorine alkaloid from *Lycoris radiata* (Amaryllidaceae) (Nagakawa *et al.* 1956), several other alkaloids have been isolated from the family (Bin *et al.*, 2013; Hulcova *et al.*, 2018). The *H. amaryllis* is equally rich in lycorine and tazetine alkaloids, often constituting 0.5% of the plant's weight. Lesser amounts of alkaloids are present in the flower and bulbs of the plant.

Certain medicinal plants produce large amounts of secondary metabolites with diverse chemical structures and functions (Hulcova *et al*., 2018). Some of the metabolites act as UV filters, antioxidants, anti-aging (Godwin *et al*., 2022), anti-inflammatory (Morakinyo *et al*., 2022), and antimicrobials when consumed by humans (Evurani *et al.*, 2019). Considering their clinical importance, several of the metabolites have been isolated and used as pharmaceutical active ingredients in many drugs (Haidan *et al.*, 2016). Alkaloids of *H. amaryllis* have been reportedly used in the treatment of centrally mediated inflammatory-related conditions such as depression, seizures and anxiety.

Inflammation is a defensive response of the immune system to damaging *stimuli* which could be pathogens or non-pathogens (Chen *et al*., 2018). Pathogenic agents include bacteria, fungi, viruses or parasites. Non-pathogenic agents include chemicals, drugs, ultra violet radiation, malnutrition and stress (Achoui *et al*., 2010). The pathogen-associated inflammatory responses are activated when a pathogen infects the body and trigger immune response. The non-pathogen associated inflammatory response occurs in response to tissue injury, cell death, cancer etc. Both agents may trigger acute or chronic inflammatory responses leading to diverse tissue injury (Derk *et al*., 2009). Uncontrolled inflammation is the foundation of several diseases and disorders irrespective of the etiology (Iwalewa *et al*., 2007). Steroidal and nonsteroidal anti-inflammatory drugs are employed for treatment of inflammation (Bertram, 2004; Akinpelu *et al*., 2018). However, their uses in inflammatory conditions are limited due to adverse effects (Harirforoosh *et al*., 2013). Therefore, new drug alternatives with little or no side effects are urgently needed (Greaves, 1976). The use of natural products in inflammatory conditions is well reported (Achoui *et al*., 2010; Morakinyo *et al*., 2022). Alkaloids from *Hippeastrum* genera had exhibited neuro-protective (López *et al*., 2002); cytotoxic and anti-malarial (Campbell *et al*., 1998), and anti-tumor (Mridul *et al*., 2018) activities. However, the anti-inflammatory activity of *Hippeastrum amaryllis* flower on bovine erythrocyte membrane stabilization and inhibition of tissue protein denaturation has not been reported; hence, this study.

Materials and Methods

Collection and Identification of Plant Sample

Fresh samples of *H. amaryllis* flower were collected in May 2021 at the Obafemi Awolowo University (OAU) Campus, Ile-Ife, Nigeria. The samples were identified at the IFE HERBARIUM, Department of Botany, OAU, Ile-Ife, Nigeria.

Flower Extract Preparation

The flower samples were air dried for two weeks and then pulverized using laboratory manual grinder. The powdered sample (200 g) was extracted with 80% (v/v) methanol at room temperature for 24 hrs and filtered severally with cotton wool. Thereafter, the filtrate was collected and filtered using a Whatman No. 1 filter paper and concentrated *in vacuo* to obtain the methanol crude extract.

Solvent Partitioning of *H. amaryllis* Crude Extract

The methanol crude extract of *H. amaryllis* was partitioned following the method of Ajayi *et al.* (2019). Prior to partitioning, the n-hexane and DCM solvent systems were separately saturated with equal volumes of distilled water in a separatory funnel. Thereafter, a portion of the methanol crude extract was suspended in distilled water and partitioned with *n*-hexane in the separatory funnel. The *n*- hexane layer was collected into a beaker and the process was repeated 3 times until the *n*-hexane layer was completely removed. The residue was further partitioned with DCM to obtain the DCM fraction. The *n*-hexane and DCM solvent fractions were separately concentrated *in vacuo* to obtain the *n*-hexane and DCM fractions, respectively.

Anti-inflammatory Assays

Preparation of 2% (v/v) Bovine Red Blood Cells

Fresh bovine blood was collected at an abattoir in Ile-Ife, Osun, Nigeria in November 2021. The sample was collected into a clean bottle containing 3.8% (w/v) trisodium citrate as anti-coagulant. The blood sample was transferred into clean centrifuge tube and centrifuged at 3000 rpm for 10 min. The supernatant containing the plasma was carefully removed using Pasteur pipette. The pallet (packed erythrocytes) was re-suspended in freshly prepared isosaline and centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed and the process was repeated until a clear supernatant layer was obtained. Then 2 mL of the packed erythrocytes were made up to 100 mL with isosaline to make 2%(v/v) red blood cells (Oyedapo *et al.*, 2010).

Membrane Stabilization Assay

The membrane stabilisation assay was carried out as described by Oyedapo *et al.* (2010). The assay mixture consisted of 1.0 mL of hyposaline (0.25% w/v NaCl), 0.5 mL phosphate buffer (0.15 M, pH 7.4), varying concentrations of the extract or drug (0.350 µg/mL), which was made up to 3.0 mL with normal saline (0.85% w/v NaCl) and 0.5 mL of 2% (v/v) red blood cells. The blood control was prepared as above but without the drug; while the drug control was prepared without the 2% (v/v) rbc. Diclofenac sodium was used as the reference drug. The reaction mixture was incubated at 56 ^{o}C for 30 min and centrifuged at 3000 rpm for 10 min. The supernatant was collected and the absorbance was measured at 560 nm against the reagent blank. The experiment was performed in triplicates and percentage membrane stability was calculated from the expression:

% stability= [100-(Abs of test drug-Abs of drug control)]/Abs of blood control × 100
The blood control without the drug represented 100% lysis.

Inhibition of Albumin Denaturation Assay

The inhibition of albumin denaturation was determined (Mizushima and Kobayashi, 1968; Aina *et al.,* 2013). The inhibition of protein denaturation was carried out using bovine serum albumin as standard protein and diclofenac sodium as the reference drug. The reaction mixture consisted of 0.5 mL (BSA, 0.25 mg/mL) and varying concentrations of the extract or drug (0 - 400 µg/mL) to a volume of 3.0 mL. This was incubated at 37 ^{o}C for 20 min and then heated at 57 ^{o}C for 30 min. The mixture was allowed to cool before addition of 2.5 M phosphate buffer (0.5 M, pH 6.3). Thereafter, 1.0 mL of the reaction mixture was pipetted into a clean test tube followed by addition of alkaline copper reagent (1.0 mL) and 1.0 mL of Folin-Ciocateu's reagent (10%). The reaction mixture was incubated at 55 ^{o}C for 10 min and allowed to cool. Absorbance of the intact protein was measured at 650 nm against the reagent blank. The percentage anti-denaturant activity was calculated from the expression.

$$\% \text{ Inhibition} = \frac{Absorbance\ of\ test - Absorbance\ of\ control}{Absorbance\ of\ test} x100$$

GC-MS Analysis of the n-Hexane Fraction of *H. amaryllis*

The GC-MS analysis was carried out at the Afe Babalola University, Ekiti State, Nigeria. The sample was subjected to chromatographic analysis on a Varian 3800/4000 gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column VF-5MS fussed silica capillary column (30.0 m x 0.25 mm x 0.25 µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70 ev; nitrogen (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 0.5 µL was employed (split ratio of 10:1) injector temperature 240 ^{o}C ion-source temperature 200 ^{o}C. The oven temperature was programmed from 70 ^{o}C (isothermal for 3 min), with an increase of 10 ^{o}C/min, to 240 ^{o}C, ending with a 9 min isothermal at 280 ^{o}C. Mass spectra were taken at 70 ev; a scan interval of 0.5 seconds and fragments from 40 to 1000 Da. Total GC running time was 42 min.

Compound Identification in the GC-MS Analysis

The identification of the various components of the sample was based on comparison of their mass spectra with the internal reference stored in the NIST Library.

Data Analysis

The data from the study was analysed using the Microsoft Excel 2013 Package. Differences between the mean values were determined using one-way analysis of variance (ANOVA)

by Duncan multiple range test. Values were expressed as Mean ± SEM (n = 3).

Results and Discussion

Results

The membrane stabilizing activities of *H. amaryllis* flower extract and fractions are presented in Figure 1. The results were compared with diclofenac sodium as a reference. Both the extract and fractions inhibited erythrocyte membrane lysis at various concentrations tested and competed favourably with diclofenac sodium (84.78 ± 0.003%). The percentage membrane stabilizing activity of *n*-hexane fraction (81.26 ± 0.00%) was significantly higher than DCM fraction (20.14 ± 0.00%) and the crude extract (15.57 ± 0.00%) respectively. At concentrations higher than 200 µg/mL, the crude extract was found to lyse the stressed erythrocytes. The mode of protection of both the crude and fractions was biphasic; while the reference drug was monophasic. The anti-denaturant activity of both the crude extract and fractions of *H. amaryllis* flower on bovine serum albumin is presented in Figure 2. The percentage inhibition of *n*-hexane fraction (93.62 ± 0.01%) was significantly higher than the DCM fraction (72.91 ± 0.001%). There was no significant difference between the percentage inhibition of *n*-hexane and diclofenac sodium (86.20 ± 0.00%).

The GC-MS analysis showed that *n*-hexane fraction of *H. amaryllis* flower was composed of fifteen compounds; with four of the compounds constituting 53.73% (Figure 4; Table 1). The major constituents of *H. amaryllis n*-hexane fraction are oleic acid (16.39%), campesterol (14.34%), *n*-hexadecanoic acid (13.68%), and 11-octadecenoic acid, methyl ester (9.32%) (Figure 3, and Table 4.1). Oleic acid (16.39%) had the highest percentage composition and is therefore considered the predominant constituent of *H. amaryllis* flower. In contrast, the 1-hydroxy-3-methyl-2-butanone (1.02%) and 4-phenylbut-3-ene-1-yne (1.05%) were the minor components constituting less than 3% of the *H. amaryllis* flower.

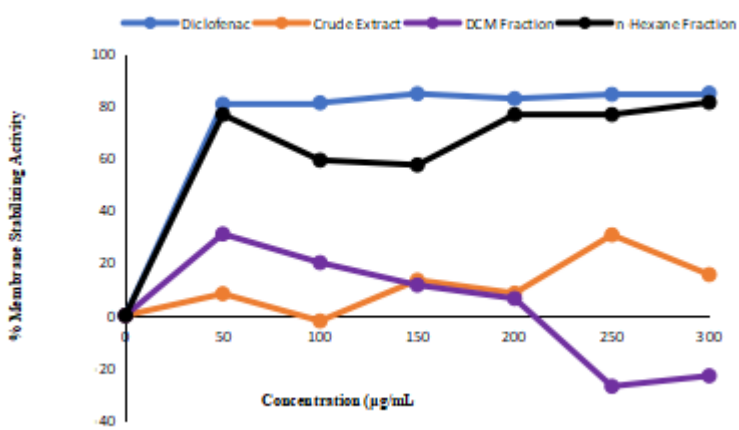


Figure 1: Percentage erythrocytes membrane stabilization activity of H. amaryllis flow

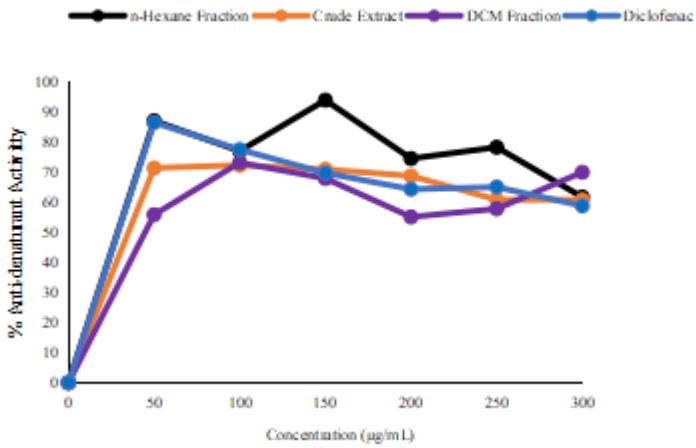
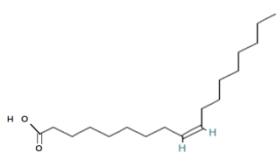
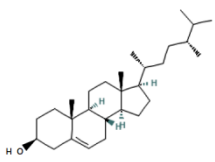


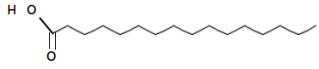
Figure 2: Percentage inhibition of albumin denaturation of *H. amaryllis* flower.



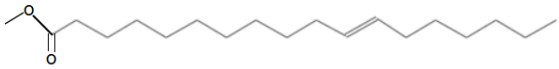
Oleic acid



Campsterol



n-hexadecanoic acid



11-Octadecenoic acid, methyl ester

Figure 3: Chemical structures of prominent compounds present in *H. amaryllis* flower.

Table 1: Compounds Identified in *H. amaryllis* Flower *n*-Hexane Fraction by GC-MS

Peak #	**RT**	**Compound Detected**	**Mol. Formula**	**Peak Area %**	**% Match (m/z)**
1	7.50	2-Butanone	C_4H_8O	6.15	72
2	7.81	1-Hydroxy-3-methyl-2-butanone	$C_5H_{10}O_2$	1.02	71
3	12.00	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	13.68*	73
4	13.00	4-Phenylbut-3-ene-1-yne	$C_{10}H_8$	1.05	51
5	17.00	Oleic acid	$C_{18}H_{34}O_2$	16.39*	55

6	18.11	Hexadecanal	$C_{16}H_{32}O$	4.10	82
7	21.50	Limonene oxide, cis-	$C_{10}H_{16}O$	4.61	55
8	22.00	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	6.14	73
9	25.00	11-Octadecenoic acid, methyl ester	$C_{18}H_{34}O_2$	9.32*	55
10	27.50	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	7.17	55
11	28.50	Cis-vaccenic acid	$C_{18}H_{34}O_2$	4.23	55
12	30.50	Octadecanoic acid	$C_{18}H_{36}O_2$	6.15	73
13	31.75	Erucic acid	$C_{22}H_{42}O_2$	2.56	55
14	40.48	Lup-20(29)-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	3.07	43
15	44.56	Campesterol	$C_{28}H_{48}O$	14.34*	56

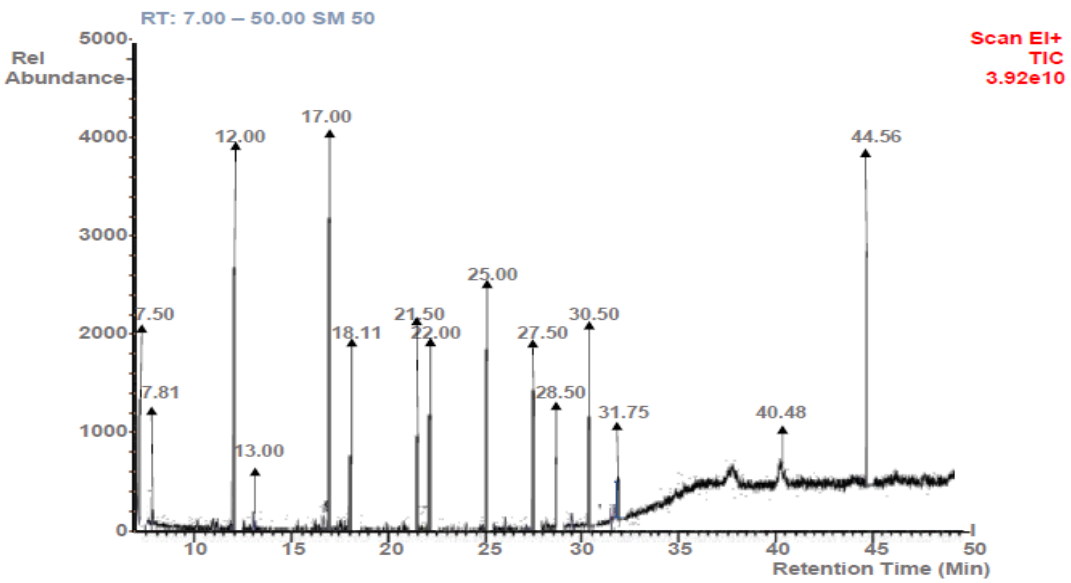


Figure 4: GC-MS chromatogram of *H. amaryllis* n-hexane fraction showing its chemical constituents.

Discussion

This present study investigated the anti-inflammatory activity of *H. amaryllis* flower extract and fractions *in vitro*. The goal of inflammatory response is to arrest, destroy, localize and remove damaged tissue components including the *stimuli* and restore normal homeostatic functions (Artis and Spits, 2015). In this study however, membrane stabilization and anti-

denaturant models of inflammation were adopted to access the anti-inflammatory potential of *H. amaryllis* flower extract and fractions. Results showed that only the *n*-hexane fraction produced significant membrane stabilizing activity at 50 µg/mL which competed favourably with diclofenac sodium at 300 µg/mL. The crude extract and the DCM fraction elicited weak percentage erythrocyte membrane stabilization across the various concentrations tested.

Membrane stabilization is the process by which healthy cells preserve the integrity of their membrane against external aggression or harm (Anosike *et al*., 2019). Determination of the membrane stabilization potential of an extract is essential because the health of a cell is dependent on the membrane integrity. Agents that stabilize cell membrane are known to inhibit early phase of inflammation. The action of *H. amaryllis* flower extract in stabilizing the bovine erythrocyte membrane could be attributed to the presence of constituents which interacted with the red blood cell membrane and altered the charges on their membrane (Chikezie *et al*., 2010; Marrassini *et al*., 2018). Some phytochemicals such as polyphenols, flavonoids, and saponins have been reported to elicit membrane stabilizing effects on bovine erythrocytes (Oyedapo *et al.,* 2010; Akinpelu *et al.,* 2018). Therefore, the ability of *H. amaryllis* flower extract to protect the erythrocyte membranes against heat and hypotonic induced lysis could be considered as anti-inflammatory mechanism.

Furthermore, the *n*-hexane fraction of *H. amaryllis* flower extract also demonstrated significant anti-denaturant activity comparable with the diclofenac sodium. The fraction showed highest anti-denaturant activity at the lowest concentration (50 µg/mL) tested. As the concentration was increased, the amount of protection was significantly reduced.

The fever (heat) and loss of tissue function in inflammatory conditions have been attributed to tissue protein denaturation (Mizushima and Kobayashi, 1968). Denaturation and inflammatory disorder exhibit similar physio-pathology and both could be produced by heat, radiations, organic solvents, etc. (Mizushima and Kobayashi, 1968). NSAIDs used in the management of inflammatory conditions have been reported in the protection of tissue proteins against denaturation (Mizushima and Kobayashi, 1968). Protein denaturation inhibitory capacity of *H. amaryllis* flower extract observed in this study, supports anti-inflammatory property of the plant.

The result of the GC-MS analysis showed that the *n*-hexane fraction of *H. amaryllis* flower composed of mainly fifteen (15) compounds in which four (4) of the constituents make up 53.73% of the total constituents. The major constituents identified in the flower sample include oleic acid, campesterol, *n*-hexadecanoic acid, and 11-octadecenoic acid, methyl ester. Oleic acid (an omega 9 fatty acid) had highest percentage composition in the *n*-hexane fraction and was considered as the most abundant constituent. Diets rich in oleic acid (also known as anti-inflammatory fat) have been reported to have beneficial effects in the control of inflammatory conditions through the activation of competent immune cells (Carrillo *et al*., 2012). Campesterol is a phytosterol abundant in many plants which have long history of anti-inflammatory activity (Begona *et al*., 2016). The reported biological

activities of the major constituents identified in *H. amaryllis* flower significantly support the findings of this study.

Conclusions

In conclusion, the study shows that *H. amaryllis* flower extract possessed anti-inflammatory property attributable to the presence of oleic acid, *n*-hexadecanoic acid, and campesterol identified in the plant sample. The mechanism of the anti-inflammatory process could be via synergistic or addictive interactions of the individual constituents present in the *H. amaryllis* extract.

References

Achoui, M., Appleton, D., Abdulla, M. A., Awang, K. and Mohd, M. A. (2010). *In vitro* and *in vivo* anti-inflammatory activities of 17-*O*-Acetylacuminolide through the inhibition of cytokines, NF-kB translocation and IKKβ activity. *Plos One,* 5(12): e15105.

Aina, O. I. and Oyedapo, O. O. (2013). *In vitro* investigations into the antioxidant and anti-inflammatory potentials of the fractions and ethanolic extract of *Cyclosorus afer* (Christ.) Ching, stalks. *Ife Journal of Science*, 15 (2): 235-249.

Ajayi, O. S., Aderogba, M. A., Obuotor, E. M. and Majinda, R. R. T. (2019). Acetylcholinesterase inhibitor from *Anthocleista vogelii* leaf extracts. *Journal of Ethnopharmacology*, 231: 503-506.

Akinpelu, B. A., Godwin**,** A., Aderogba, M. A., Makinde, A. M., Azeez, S. O. and Oziegbe M. (2018). Evaluation of anti-inflammatory and genotoxicity potentials of the fractions of *Archidium ohioense* (Schimp. ex Mull) extract. *Ife Journal of Science*, 20(3): 487-495.

Anosike, C. A., Odinaka, N. I. and Nwodo, O. F. C. (2019). Antioxidant properties and membrane stabilization effects of methanol extract of *Mucuna pruriens* leaves on normal and sickle erythrocytes. *Journal of Traditional and Complementary Medicine*, 9(4): 278-284.

Artis, D. and Spits, H. (2015). The biology of innate lymphoid cells. *Nature,* 517: 293–301.

Begona, M.M., Sabater-Jara, A.B., Pedreno M.A. and Almagro, L. (2016). Bioactivity of phytosterols and their production in plant *in vitro* cultures. *Journal of Agricultural Food Chemisty* 64(38): 7049-58.

Bin H., Shu-Fang S. and Qing-Jie Z. (2013). Cytotoxic and antimalarial Amaryllidaceae alkaloids from the bulbs of *Lycoris radiate*. *Molecules*, 18: 2458-2468.

Campbell, W.E., Nair, J.J., Gammon, D.W., Bastida, J., Codina, C., Viladomat, F., Smith, P.J., and Albrecht, C.F. (1998). Cytotoxic and antimalarial alkaloids from *Brunsvigia littoralis. Planta Medica,* 64: 91–93.

Carrillo, C. M., del Cavia M. and Alonso-Torre, S. (2012). Role of oleic acid in immune system; mechanism of action, *Nutricion Hospitalaria,* 27(4): 978-990.

Chen, D., Holly, S., Ana, G., Tsutomu, A., Shamra, M., Charlotte, V., Chiao-Wen, C.,

Xiaoqin, F., Anne, W., Wei, B., Liang, L., Lisa, F., Kurt, J., and Yvonne, R. F. (2018). Inhibition of toll-like receptor-mediated inflammation *in vitro* and *in vivo* by a novel Benzoxaborole. *The Journal of Pharmacology and Experimental Therapeutics*, 344: 436-446.

Chikezie P. C., Uwakwe A. A. and Monago C. C. (2010). Comparative osmotic fragility of three erythrocyte genotypes (*HbAA*, *HbAS* and *HbSS*) of male participants administered with five antimalarial drugs. *African Journal of Biochemical Research*, 4(3): 57–64.

Cimmino, A., Masi, M., Evidente, M., Superchi, S. and Evidente, A. (2017). Amaryllidaceae alkaloids: absolute configuration and biological activity. *Chirality*, 29(9): 486-499.

Derk, A., Karin, E. D., and Terrence, T. (2009). Approaches to determine expression of inflammatory cytokines. *Methods in Molecular Biology*, 511: 107–142.

Evurani S. A., Godwin A., Charity G., Chinelo E. C., Okafor G. (2019). Antimicrobial activity of lactic acid bacteria from Kunun-Zaki on selected clinical pathogens. *South Asian Journal of Research in Microbiology*, 3(4): 1-8.

Godwin, A., Ojelabi, O.M., Romanus, M.R., Makinde, B.I., Aderogba, M.A. and Akinpelu, B.A. (2022). Preliminary investigation of skin protection potential of partially purified leaf fraction of *Asystasia vogeliana* Benth. *Adeleke University Journal of Science*, 1(1): 1-11.

Haidan, Y., Qianqian, M., Li, Y. and Guangchun, P. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21(559): 1-18.

Hulcova, D., Breiterova, K., Siatka, T., Klimova, K., Davani, L., Safratova, M., Hostalkova, A., De Simone, A., Andrisano, V. and Cahlikova, L. (2018). Amaryllidaceae alkaloids as potential glycogen synthase Kinase-3beta inhibitors. *Molecules*, 23 (4): 719.

Iwalewa, E. O., McGaw, L. J., Naidoo, V. and Eloff, J. N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology*, 6(25): 2868-2885.

López, S., Bastida, J., Viladomat, F. and Codina, C. (2002). Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and Narcissus extracts. *Life Sciences, 71*, 2521–2529.

Marrassini, C., Peralta, I. and Claudia, A. (2018). Comparative study of the polyphenol content‐related anti‐inflammatory and antioxidant activities of two *Urera aurantiaca* specimens from different geographical areas. *Chinese Medicine*, 13(22): 2-12.

Mizushima, Y. and Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, 20: 169-173.

Morakinyo A.E., Godwin A., Akwatang M.D., Ikulala O.O., Abdulsallam K. A., Nzekwe S.C., Oyedapo O.O. and Oyedepo T. A. (2022). Effects of Heat and Fermentation on the Anti-inflammatory and Antioxidant Activities of *Ricinus communis* (Castor) Seeds. *Egyptian Academic Journal of Biological Sciences, H. Botany,* 13(1): 79-92.

Mridul R., Long L., Xiaojuan X., Fenga P., Mao Y. and Jing L. (2018). Lycorine: A prospective natural lead for anticancer drug discovery. *Biomedicine and Pharmacotherapy,* 107; 615-624.

Nair, J.J., Wilhelm, A., Bonnet, S.L. and van Staden, J. (2017). Antibacterial constituents of the plant family Amaryllidaceae. *Bioorganic & Medicinal Chemistry Letters*, 27(22); 4943-4951.

Oyedapo, O.O., and Famurewa A. J. (2010). Antiprotease and extracts of *Fagara zanthoxyloides, Olax subscorpoiodes* and *Tetrapleura tetraptera*. *International Journal of Pharmacognosy*, 33(1): 65-69.