Journal of Medicinal Herbs
journal homepage:**www.jhd.iaushk.ac.ir**

A furostan saponin isolated from the rhizome of *C. spectabilis* (Costaceae) exerts cataract ameliorative effect *in-vitro*

Salisu Shehu[*1], Umar Habib Danmalam[1], Najma Ilyas[1], Mohammed Danjuma[2], Aliyu Musa[3]

[1]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Nigeria;*
Email:** ***salisshe18@gmail.com

[2]*Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria;*
[3]*Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University Zaria, Nigeria;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *September 21th2022*
Accepted *January 29th2023*

Key words:
- ✓ *Column chromatography*
- ✓ *Cataract*
- ✓ *Bio-molecules*
- ✓ *Opacity*
- ✓ *Antioxidant*

ABSTRACT

Background & Aim: Extracts from plants, such as *C. spectabilis* (rhizome) traditionally used for the treatment of cataract may potentially contain anticataract lead compound(s). The current study aimed to isolate the constituent(s) of the extract of *C. spectabilis* and evaluate its anticataract effect.

Experimental: The isolation of the constituent was achieved using silica gel, sephadex column chromatography and preparative thin layer chromatography of the n-butanol fraction of aqueous ethanol extract of the rhizome, followed by analysis using NMR spectroscopy. The anticataract effect was investigated using H_2O_2-induced cataract model. Lenses freshly obtained from rats were cultured in the presence or absence of hydrogen peroxide (0.5 mM), and or in the presence of H_2O_2 (0.5 mM) with any of the three concentrations of compound SL1 (0.5 $mgmL^{-1}$, 0.25 $mgmL^{-1}$, or 0.125 $mgmL^{-1}$) over a period of 24 hrs. Lens opacity (index of cataract) was quantified by scoring and image analysis. The lens total protein, antioxidant bio-molecules (GSH, SOD) and lipid peroxidation (MDA level) were determined according to standard methods.

Results: Chromatographic fractionation of the extract led to the isolation of a compound characterised as 3-O-α-L-rhamnopyranosyl (1→2)-α-O-β-D-glucopyranoside (25R)-furost-5-ene-3β, 22α, 26-triol-26-O- β-D-glucopyranoside based on its NMR data. The lenses treated with hydrogen peroxide (only) demonstrated significantly higher indices of opacity compared to the normal or compound treated. The groups treated with the compound (at 0.5 and 0.25 $mgmL^{-1}$ concentrations) significantly ($P \leq 0.001$) exhibited lower score of opacity and grey image pixel intensity compared to the untreated group. Although the compound significantly ($P \leq 0.05$) prevented the depletion of lens total protein at all concentrations used, the loss of GSH, SOD and increase in MDA levels induced by H_2O_2 were not prevented significantly.

Recommended applications/industries: The compound isolated can serve as a promising lead for the development of anticataract drug.

1. Introduction

Cataract is a pathologic condition of the eye that is characterized by opacification of the lens, reduced transparency of the lens, decreased visual acuity and finally lead to blindness if not treated. According to World Health Organization report, cataract account for 65.2 million cases of visual impairment in the world (WHO, 2019).

The most widely used clinical intervention employed in the management of cataract involves the surgical removal of the cataractous lens and replacement with an artificial intraocular lens. Hence, the search for pharmacological alternative that could likely be cheaper and more accessible for the prevention and treatment of this disorder is receiving much attention in ophthalmology research (Vuanghao *et al.*, 2018). Several studies have indicated the potential role of many plant extracts or their isolated compounds against cataract formation (Vuanghao *et al.*, 2018). *Costus spectabilis* (Fenzl) K. Schum. of the Costaceae family often referred to as an ornamental *Costus*, is a rhizomatous geophyte native to tropical Africa, commonly called Yellow trumphet. Its underground storage organs are rhizomes and have been employed for treatment of various illnesses in African traditional medicine. Consequent to its folkloric use in management of cataract earlier reported (Shehu and Dauda, 2018), It has recently been investigated and demonstrated to exert anticataract effect *in-vitro* (Shehu *et al.*, 2019).

In the current study an attempt was made to isolate and test the anticataract potential of a chemical constituent of the extract of the rhizome of *C. spectabilis*.

2. Materials and Methods

2.1. Materials

Glass columns, Silica gel (60-120 mesh) for column chromatography (Merck Germany), Pre-coated thin layer chromatography (TLC) glass plates (Silica gel 60 F254, thickness 1 mm) for preparative TLC (Merck Germany), Pre-coated TLC aluminium plates (Silica gel 60 F254) for TLC analysis (Merck Germany), Bruker AVANCE-600 Japan (600MHz) and Rotary evaporator (R-II-HB, Switzerland). The chemicals used were of analytical grade and were obtained from Sigma Aldrich St. Louis, MO, USA and Qualikems fine chem., Nandesari, India. The collection of the plant material was performed on the month of September, 2018, from Shika, Zaria, Nigeria. It was later identified at the Bioresources Unit, National Research Institute for Chemical Technology (NARICT) Basawa Zaria with the aid of a voucher specimen No. 1611. The rhizome of the plant material was then collected, dried and pulverized.

2.1.1. Animals

Wistar rats of both sexes (150-200g) obtained from the animal house stock of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria were used for the study. The study was carried out in accordance with the guidelines and approval of Animal use and care committee of Ahmadu Bello University Zaria with an approval number ABUCAUC/2017/006.

2.2. Methods

2.2.1. Extraction and isolation of the compound

The powdered plant material (470g) was macerated in 5 L of 70% ethanol in water over 72 h. About 40 g of the extract obtained after concentration on rotary evaporator was later suspended in water and extracted successively with n-hexane, chloroform, ethyl acetate and n-butanol, where 0.85, 8.1, 0.4 and 7.7 g of the partition fractions were obtained, respectively.

The n-butanol fraction (4 g) was fractionated using silica gel column chromatography by gradient elution method, which was started with chloroform/ethylacetate (1:1) and then with methanol 5% , 10% , 50 % and 100 % in an increasing polarity to a total of 123 column fractions collected. Column Fractions 99-120 having similar TLC profile containing the most significant amount of the fraction of the extract were pooled (0.2 g) and labeled as CF.

2.2.2. Purification of column fraction (CF)

The column fraction (CF) was purified over Sephadex LH-20 column. The elution was performed isocratically using methanol, which led to separation of 15 column fractions. Column fractions 5 to 7 being of similar profile were pooled and labeled as CFA. Finally, CFA was purified by preparative thin layer chromatography with silica gel (60 F254, 1 mm thick) as the stationary phase and the lower layer of chloroform/methanol/water (6.5:3.5:1) as the mobile phase, and this afforded the isolation of compound SL1 (10 mg).

2.2.3. Spectroscopic analysis of the isolated compound SL1

Compound SL1 was subjected to 1D and 2D-Nuclear magnetic resonance (NMR) spectroscopy at School of Physics and Chemistry, University of Kwazuulu Natal, South Africa. The study included 1D

(^{1}H, APT ^{13}C) and 2D [COSY (Correlated spectroscopy), HSQC (Heteronuclear single quantum correlation) and HMBC (Heteronuclear multiple bond correlation] spectroscopy.

2.2.4. In-vitro anticataract studies

2.2.4.1. Lens collection and culture

The Freshly dissected lenses were isolated through a posterior approach from the eyeball of the rats that were initially anaesthetised using chloroform and sacrificed. Adherent vitreous materials were then removed by rolling the lenses on filter paper.

The lenses were then transferred to culture plate (Falcon plastic), one in each well containing 1 mL of the culture medium prepared as described by Lokesh (2015). The composition of the medium includes: NaCl (140 mM), KCl (5 mM), $MgCl_2$ (2 mM), $NaHCO_3$ (0.5 mM), NaH $(PO4)_2$ (0.5 mM), $CaCl_2$ (0.4 mM), streptomycin (100 µg/mL), penicillin (100 IU/mL) and a pH of 7.8. The lenses were then incubated at room temperature for 2 hours and examined. All lenses that are not transparent were excluded from the study.

2.2.4.2. Hydrogen peroxide-induced cataract

Hydrogen peroxide-induced cataract model was conducted as described by Suchita *et al*. (2015). The study involved five groups; the normal, control (toxic) and three test groups, where each group is comprised of 5 lenses. While the lenses in the normal group were cultured in the medium described earlier, the control (toxic) and the three test groups were cultured in the same medium, but incorporated with hydrogen peroxide (0.5 mM). In addition, the media of the 3 test groups were added with 0.5 $mgmL^{-1}$, 0.25 $mgmL^{-1}$ or 0.125 $mgmL^{-1}$ concentrations of the compound. Lenses in all the groups were then incubated at room temperature for 24 hours and subsequently assessed for opacity. The examination of lens opacity was conducted in accordance with Shruthi *et al*. (2012). Lens opacity was scored as described by Dickerson *et al*. (1997). Where; 0, 0.2, 0.5, and 1 was assigned for a transparent, spots containing, cloudy and opaque lens respectively. The images of all lenses were then captured using Olympus DP20 camera, converted in 16-bit gray scale images and their mean pixel intensities determined using Image j (developed by NIH). High pixel intensity value indicates lens opacity (Shruthi *et al.*, 2012).

2.2.4.3. Evaluation of lens bio-molecules

Total protein (TPC)

The lens total protein was determined according to the method of Lowry *et a*l. (1951). The study commenced by addition of 2 mL of alkaline $CuSO_4$ to 0.2 mL solution of lens homogenate. Folin Ciocalteau solution (0.2 mL) was then added and mixed thoroughly. About 30 minutes later, the absorbance was measured at 660 nm. Similar procedure was repeated using bovine serum albumin as the standard.

Reduced glutathione (GSH)

The evaluation of reduced glutathione (GSH) level was done by adopting the method of Rajagopalan *et al*. (2004). The lens homogenate (in PBS, pH 7.4) measuring 150 µL, was mixed with 10% trichloroacetic acid (1.5 mL) and centrifuged. Exactly, 1 mL of the supernatant was then added with 0.5 mL of Ellman's reagent and 3 mL of 0.2 M phosphate buffer (pH 8.0). At 412 nm the absorbance of the mixture was then determined.

Malondialdehyde (MDA)

The MDA level was determined in accordance with the method described by Akanji *et al*. (2009). Two milliliter (2 mL) of a mixture of 15%Trichloroacetic Acid, 0.37% Thiobarbituric Acid, 0.25 N HCL (1:1:1) was added with 150 µL of the lens homogenate, then placed in a water bath at 90°C for 60 minutes, cooled and centrifuged. At 535 nm, the absorbance of the supernatant complex was measured. Using the molar extinction coefficient, the MDA level was determined.

Superoxide Dismutase (SOD)

The estimation of superoxide dismutase (SOD) was performed based on a previous protocol (Fridovich, 1989). The lens homogenate (0.1 mL) was diluted in 0.9 ml distilled water. Then, 2.5 mL of 0.05 M carbonate buffer was added to 0.2 mL of the diluted microsome. The mixture was then added with 0.3 mL of 0.3 mM adrenaline. Distilled water (0.2 mL) was used to instead of lens homogenate in the reference mixture. At 480 nm, the absorbance was measured over 30- 150 seconds. Using the standard relationship, the SOD level was then determined.

2.3. Statistical analysis

Data were presented as mean (± SEM)) and analyzed by one way ANOVA using SPSS 20.0. Difference between means at $p \leq 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. NMR characterization and physicochemical properties of compound SL1

Compound SL1 was obtained as white amorphous powder, slowly soluble in methanol. Based on the informations deduced from the NMR spectra of compound SL1, the various resonances of ^{1}H and ^{13}C, HSQC, multiplicities and coupling constants were presented (Table 1).

The 1H NMR spectrum of SL1 (in MeOD/DMSO) showed two signals due to angular methyl groups of a steroid nucleus, i.e. at δH 0.83 (s, H3-18) and 1.04 ppm (s, H3-19), and 2 methyl doublets, at δH 0.88 ppm (d, J = 12.00 Hz, H3-27) and 1.01 ppm (d, J = 12.06 Hz, H3-21). These signals correlated in the HSQC spectrum of SL1 with δC 17.20, 14.60, 17.70, and 16.30 ppm, respectively. In addition, the COSY correlation between an oxymethine proton signal δH 4.36 ppm (H-16) and methine proton signal δH 1.76 ppm (H-17) suggests a furostane or spirostane steroid nucleus. The HMBC correlations of these characteristic methyl groups were used in the assignment of the steroid nucleus; the correlations of δH 0.88 ppm (H3-27) for example, allowed assignment of C-24 (δC 29.30) and C-25 (δC 30.80). The HMBC cross peaks between methyl protons at δH 0.83 ppm (H3-18) allowed the assignment of C-13 (δC 40.80), C-14 (δC 57.10), C-12 (δC 40.70) and C-17 (δC 63.10). Also, methyl proton signals at δH 1.04 ppm (H-19) shows correlation with the most de-shielded carbon signal at δC 140.6 ppm corresponding to the quaternary carbon (C-5), and this confirm the assignment of oleifenic bond between C5 and C6 suggesting Δ^5-unsaturated steroid compounds (Agrawal *et al.*, 1985). The proton signal at δH 3.37 and 3.75 were assigned for H-26a and H-26b respectively and these showed HSQC cross peak with the relatively more de-shielded carbon signal δC 74.34 (C-26). The difference in chemical shift (Δab) between H-26a (δa) and H-26b δb (δb –δa) is 0.38 ppm (less than 0.48 ppm), which suggest (25R)-configuration (Challinor *et al.*, 2012).

The downfield signal seen at C-26 is as a result of glycosylation which is confirmed by the HMBC correlation observed between the H-26 proton signal δH 3.75 ppm and the anomeric carbon at δC 110.50, while the HMBC correlation observed between C-22 (δC 109.70) and δH 1.01 ppm (H3-21) suggests a 22-hydroxy furostanol saponin (Challinor *et al.*, 2012).

The 1H NMR spectrum of SL1 also showed three signals typical of the anomeric proton of a sugar, at δH 5.08 ppm (d, J = 5.46 Hz, H-1, 26-O- β -D-glucose), 4.53 (d, J = 9.36 Hz, H-1', 3-O- β - D-glucose) and 5.23 ppm (br s, H-1", 2-O-α-L-Rhamnose), which showed HSQC correlations with signals at δC 110.50, 99.60 and 101.30 ppm, respectively.

The HMBC spectrum of SL1 was also used to establish the position of attachment between the sugar units and between the sugars and the aglycone. For example, the cross peak observed between δH 5.08 (H-1, 26-O- β -D-glucose) and δC 74.40 (C-26, aglycone) revealed the attachment of a single glucose residue at C-26, commonly found in furostanol saponins. From the data of the spectra of SL1, two sugar units, glucose and rhamnose, were suggested to be at C-3 position of the steroid aglycone. The suggestion is supported by the HMBC correlations observed between δH 4.53 (H-1', 3-O- β -D-glucose) and δC 76.90 (C-3, aglycone) and δH 5.23 (H-1, 2-O- α-L-Rhamnose) and δC 72.20 (C-2, 3-O- β -D-glucose) which allowed identification of the 1→2 linked glucose→rhamnose units. The COSY spectrum was used in the assignment of various carbon atoms of the sugar molecules. For example, the COSY relationship between δH 5.08 (H-1, 26-O- β -D-glucose) of the anomeric carbon and δH 3.89 (H-2" ') of the second carbon atom of the sugar molecule, and between δH 4.53 (H-1', 3-O- β- D-glucose) of the anomeric carbon of sugar molecule at C-3 and δH 3.39 (H-2') of its second carbon.

Based on the analysis of the spectral data of compound SL1 (Table 1), its COSY, HMBC spectra and by comparison with the spectral data of some compounds in the literature, compound SL1 was proposed to be 3-O- α-L-rhamnopyranosyl (1→2) - α-O-β-D-glucopyranoside (25R)-furost-5-ene-3β, 22α, 26-triol-26-O- β-D-glucopyranoside. The current work is the first to report the isolation of a furostanol compound from this plant. However, closely related compounds, methyl protodeltonin and 3-O- α -L-Rhamnopyranosyl (1→4)-α-O-β-D-glucopyranoside (25R)-furost-5-ene-3-,22α,26-triol-26-O-β-D

glucopyranoside were previously reported from different plants (Hayes *et al.*, 2007; Khodakov *et al.*, 1996). Members of the monocotyledonous families, including *costaceae* are widely known to accumulate steroidal saponins (Sobolewska *et al.*, 2020). Furthermore, furostanol saponins have been reported to be among the most important characteristic chemical principle in *Tribulus terrestris* (Hekmat *et al.*, 2019).

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of aglycone and the glycones of Compound SL1 (600 Hz, MeOD/DMSO).

C	^{13}C	^{1}H, (J-Hz)
1	37.4	1.10 d
2	31.8	1.61m
3	76.9	3.94 d (5.40)
4	40.3	2.32 m, 2.51 m
5	141.3	-
6	122.1	5.43 d (6.66)
7	32.2	1.59 m
8	32.1	1.80 m
9	50.9	1.00 d
10	37.9	-
11	21.4	1.59 m
12	40.7	1.19 m
13	40.8	-
14	57.1	1.19 m
15	32.5	1.27 m
16	81.4	4.36 m
17	63.1	1.76 m
18	17.2	0.83 s
19	14.6	1.04 s
20	42.2	1.94 dd
21	16.3	1.01 d (12.06)
22	109.7	-
23	30.1	1.61 m
24	29.3	1.40 m
25	30.8	1.73 m
26	74.3	3.37 m
		3.75 m
27	17.7	0.88 d (12.00)

C	δH	J (Hz)	δC
Glucose 1			
1'	4.53 d	11.82	99.6
2'	3.39 m	-	77.2
3'	3.64 m	-	77.1
4'	3.60 m	-	79.5
5'	3.37 m	-	75.8
6'	3.58 m	-	63.1
Rhamnose			
1"	5.23b rs	-	101.3
2"	3.88 m	-	72.5
3"	3.64 m	-	72. 4
4"	3.37 m	-	76.9
5"	4.16 m	-	71.7
6"	1.25 d	10.26	19.4
Glucose 2			
1" '	5.08 d	5.22	110.5
2" '	3.89 m	-	77.1
3" '	3.64 m	-	73.3
4" '	3.20 m	-	75.7
5" '	3.37 m	-	79.9
6" '	3.50 m	-	69.0

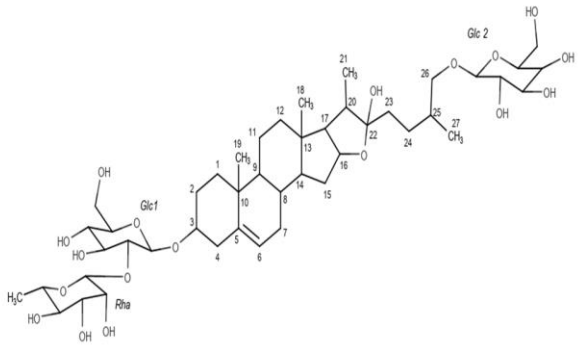

Figure 1. Chemical structure of compound SL1.

3.2. Anticataract potential of compound SL1

The findings of the anticataract studies of compound SL1 showed that, lenses in the normal control and compound treated groups appeared to be more transparent and have significantly lower cataract indices when compared with lenses in the toxic group (Figure 2, Table 2). The most significant ($P \leq 0.001$) reduction of opacity score and mean pixel intensity value was observed at 0.5 and 0.25 $mgmL^{-1}$ concentrations of the compound. The current work is the first to report the anticataract effect of a furostanol saponin. Previously, a steroidal compound, 5-cholesten-3β, 25-diol has been shown to be a promising lead towards the non-surgical treatment of both hereditary and age-associated cataracts (Makley *et al.*, 2015). Also, lanosterol has been reported to prevent cataract by significantly decreasing the preformed protein aggregates in lens, both *in-vitro* and in cell-transfection experiments (Ling *et al.*, 2015). Other steroids previously shown to exhibit anticataract effect includes lucidamol A from *Ganoderma lucidum* and 20 (S)-ginsenoside Rh_2 from Panax ginseng (Vuanghao *et al.*, 2018).

Figure 2. Image of lenses from compound (SL1) treated and control groups.

Key: CM= Normal group, P= untreated peroxide group and 0.125, 0.25, 0.5 mg/ml = the groups treated with the various concentrations of compound SL1.

Table 2. Effect of Compound SL1 on Pixel Intensity and Score of opacity of Lenses.

Treatment(mg/ml)	Mean pixel intensity ± SEM	Mean score of opacity ± SEM
CM	161.09±2.87***	0.06±0.06**
SL1 (0.5)	173.40±0.10***	0.20±0.00**
SL1 (0.25)	173.57±0.18***	0.13±0.06**
SL1 (0.125)	177.40±0.44**	0.30±0.10*
P (0.5)	189.07±1.13	0.67±0.16

Values are Mean ± S.E.M., *** = P<0.001, ** = P<0.01, * = P<0.05 compared to untreated peroxide group were considered significant. One Way ANOVA followed by Dunnett's post hoc test was used. n= 5. Key: CM = Normal group, compound SL1 (0.5), SL1 (0.25) and SL1 (0.125) groups. P (0.5) is the untreated toxic group.

3.3. Effect of compound SL1 on lens biochemical parameters

The total protein (TPC) status indicated that lenses placed in 0.5 mM H_2O_2 only, had a significant (P≤0.001) decrease of their total protein (TPC) level when compared with the normal control (Table 3). The groups treated with different doses (0.5, 0.25, and 0.125 mg/mL) of the compound had significant increase (p≤ 0.05) in the level of their total protein compared to the untreated (toxic) group (Table 3). It has been reported that; reactive oxygen species may be responsible for protein oxidation and proteolysis, which may ultimately decreases the final protein concentration of tissues during the pathophysiological conditions (Burton and Jauniaux, 2011). The GSH and SOD content of the compound treated group is relatively higher than that of the toxic group and this demonstrate a moderate effect of the compound SL1 in maintaining the levels of lens antioxidant molecules (Table 3). Previously, protodeltonin (a furostan saponin), structurally related to compound SL1, at 50µM, decreased reactive oxygen species (ROS) and increased GSH level in a H_2O_2-induced hepatotoxicity study (Siddiqui *et al.*, 2018). Although not significantly, hydrogen peroxide (0.5 mM) caused an increase in lens peroxidation. However, the malondialdehyde (MDA) observed in the toxic group were relatively higher than the normal, control and compound SL1 treated group (Table 3).

3. Effect of Compound SL1 on Lens Bio-molecules**.**

Groups	M-TPC(mg/g) ± SEM	M-GSH (mg/g) ±SEM	M-SOD (U/mg protein) ± SEM	M-MDA(µmol/mg protein) ± SEM
CM	76.11±0.14***	4.41±0.12	11.60±0.10*	64.73±0.38
SL1-0.5	74.02±0.13*	4.15±0.08	11.03±0.13	69.96±3.68
SL1-0.25	74.02±0.13*	4.28±0.27	11.00±0.00	67.63±3.50
SL1-0.125	74.30±0.55*	4.46±0.15	11.50±0.20	72.93±0.61
P-0.5	72.08±0.86	3.90±0.12	10.96±0.13	74.36±0.36

Data presented as Mean (M) ± S.E.M. *** = P<0.001 and *= P<0.05 as compared with toxic control is considered significant. One Way ANOVA followed by Dunnett's post hoc test was used. n =5. Key: CM = Normal group, compound SL1 (0.5), SL1 (0.25) and SL1 (0.125) groups. P (0.5) is the untreated toxic group.

4. Conclusion

The current study is the first to report the isolation and characterization of a furostan saponin; 3-O- α-L-rhamnopyranosyl (1→2)- α-O-β-D-glucopyranoside (25R)-furost-5-ene-3β, 22α, 26-triol-26-O- β-D-glucopyranoside from the rhizome of *C. spectabilis*, and its anticataract effect against hydrogen-peroxide induced cataract in cultured rat lenses.

5. References

Agrawal, P. K. Jain, D. C. Gupta, R. K. and Thakur, R. S. 1985. Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. *Phytochemistry* ,24 (11): 2479-2496.

Akanji, M. A.Adeyemi, O. S and Oguntoye, S. O. 2009. *Psidium guajava* extract reduces trypanosomosis associated lipid peroxidation and

raises glutathione concentrations in infected animals. *Excli Journal*, 8: 148-154.

Burton, G. J. and Jauniaux, E. 2011. Oxidative stress. Best *Practice and Research Clinical Obstetrics and Gynaecology*, 25:287–299.

Challinor, V. L. Piacente, S. and De Voss J. J. 2012. NMR Assignment of the absolute Configuration of C-25 in furostanol steroidal saponins. *Steroids*, 77: 602–608.

Dickerson, J. E. Dotzel, E. and Clark, A. F. 1997. Steroid - induced cataract: New perspective from i*n-vitro* and lens culture studies. *Experimental Eye Research*, 65: 507–516.

Fridovich, I. 1989. Superoxide dismutases. an Adaptation to a paramagnetic gas. *Journal of Biological Chemistry*, 264(14):7761-7764.

Hayes, P.Y. Lambert, L.K. Lehmann, R. Penman, K. Kitching, W. and De Voss, J. J. 2007. Spectral assignments and reference data complete 1H and 13C assignments of the four major saponins from *Dioscorea villosa* (wild yam). *Magnetic Resonance in Chemistry*, 45: 1001–1005.

Hekmat, H. Gohar, R. B. Larijani, K. 2019. Interaction of saponins extracted from *Tribulus terrestris* with human serum albumin. *Journal of Medicinal Herbs*, 10 (1): 37-46.

Khodakov, G .V. Akimov, Y.A. Shashkov, A. S. Kintia, P. K. and Grishkovets V. I. 1996. Triterpene and steroid saponins isolated from two *Melilotus species. Saponins used in Food and Agriculture*, 211–222.

Ling, Z. Xiang-Jun, C. Jie, Z. Yi-Bo, X. Xu, Y. Li-Dan, H. Hong, O. Sherrina, H. P. Xin, J., Danni, L., Frances, W., Ken, F., Huimin, C., Gen, L., Guiqun, C., Ying, L., Daniel, C., Cindy, W., Christopher, C., Yandong, W., Austin, Q., Emily, Y., Wenqiu, W., Xun, H., Seanna, G. 2015. Lanosterol reverses protein aggregation in cataracts. *Nature*, 523: 607–611.

Lokes, J.S. 2015. Evaluation of echitamine, ditamine and echitenine for anti- cataract activity using *in-vitro* cataract model. J*ournal of Applied Pharmaceutical Sciences*, 5 (5): 65-67.

Lowry, O. Rosebrough, A. Farr, A. Randall, R. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193: 265.

Makley, L. N. Kathryn, A. M. Brian, T.D, Joshua, W. G. Brittney, N. M. Ponni, R. Bryan, M. D.Thomas, J. M. Andrea, D.T. Roger, S. Rachel, E. K. Usha, P. A. and Jason, E.G. 2015. Pharmacological chaperone for Alpha-Crystallin partially restores transparency in cataract models. *Science*, 350(6261): 674–677.

Rajagopalan, R. Aruna K. V. Varma, P. S. Rajasekharan, K. and Menon, V. 2004. Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacy and Pharmaceutical Sciences*, 7: 274-283.

Shehu, S. and Dauda, B. 2018. Inventory of medicinal plants used in the treatment of eye ailments in Zaria metropolis, Kaduna State, Nigeria. *Nigerian Journal of Pharmaceutical Sciences*, 7(1):68- 75.

Shehu, S., Shehu, U. F., Shehu, S., Danmalam, U. H. and Danjuma, N. M. 2019. Extract of *Costus spectabilis* attenuates H_2O_2-Induced cataract in cultured rat lenses. *Tropical Journal of Natural Product Research*, 3 (4):145-148.

Shruthi, S., Lee Anne, M., Chiara, B., Pierre, M., Armin, W., Salah-Dine C., Francois, P., Steve, B., Nandita, S., Elba, C., Maya, G., Parul, P. and Albert, R. 2012. The use of rat lens ex-plant culture to study the mechanism of drug-induced cataractogenesis. *Toxicological Sciences*, 126(1): 128–139.

Siddiqui , M. A., Ali, Z., Chittiboyina, A., G. and Khan, I. A. 2018. Hepatoprotective effect of steroidal glycosides from *Dioscorea villosa* on hydrogen peroxide- induced hepatotoxicity in HepG2 cells. *Frontiers in Pharmacology*, 9:797.

Sobolewska, D., Galanty, A. and Grabowska, K. 2020. Saponins as Cytotoxic Agents: An update (2010-2018). Part I- steroidal saponins. *Phytochemistry Reviews*, 19: 139-189.

Suchita, D., Sudipta, S., Guarav, K. and Shubhini, A. S. 2015. Effectof standardized fruit extract of *Luffa cylindrica* on peroxide-induced cataract. *Indian Journal of Pharmacology*, 47(6): 644-648.

Vuanghao, L., Edward, S., Hongli, W. and Iok-Hou, P. 2018. Cataract preventive role of isolated phytoconstituents: Findings from a decade of research. *Nutrients* 10:1580.