World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Inhibition of advanced glycation end product and lipid peroxidation by extract and fractions of *Newbouldia laevis*

Daniel Lotanna Ajaghaku [1], Ikechukwu Sonne Mbagwu [2,*], and Amara Anwuchaepe Ajaghaku [3,4]

[1] *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Agulu, Anambra State, Nigeria.*
[3] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.*
[4] *International Institute for Pharmaceutical Research and Innovation, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.*

World Journal of Advanced Research and Reviews, 2025, 26(02), 2514-2530

Publication history: Received on 02 April 2025; revised on 10 May 2025; accepted on 12 May 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.2.1836

Abstract

Newbouldia laevis is generally known for its significant antidiabetic potentials. Advanced glycation end-products (AGEs) and lipid peroxidation contribute significantly to the pathogenesis of diabetic complications through oxidative stress and protein damage. This study evaluated the inhibitory effects of extract and fractions of *Newbouldia laevis* leaves on AGE formation and lipid peroxidation. The leaves were extracted using 70% aqueous ethanol and partitioned into n-hexane, ethyl acetate, butanol, and water fractions. *In vitro* assays revealed the ethyl acetate fraction with the highest total phenolic content and exhibited the strongest ferric reducing antioxidant power and inhibition of lipid peroxidation. The formation of AGEs and glycation-induced protein carbonylation were significantly suppressed in a concentration-dependent manner, with the ethyl acetate fraction demonstrating the most potent activity in weeks 2 – 4 at 0.4 – 1.6 mg/ml concentrations, comparable to aminoguanidine, a standard AGE inhibitor. The butanol and water fractions also showed notable inhibitory effects, whereas the n-hexane fraction exhibited the least activity. The inhibitory potential of the fractions correlated with their phenolic content, suggesting a phenol-mediated mechanism. These findings support the therapeutic potential of *N. laevis*, particularly its phenol-rich fractions, in mitigating oxidative stress and protein glycation associated with *diabetes mellitus* and its complications.

Keywords: *Newbouldia Laevis*; Advanced Glycation End-Products (Ages); Lipid Peroxidation; Diabetic Complications; Phenolic Compounds; Antioxidant Activity

1. Introduction

Diabetes mellitus is a metabolic condition marked by hyperglycemia [1]. Long term exposure to high glucose concentration in the blood is associated with numerous complications that lead to impairment of kidney, eyes, nerves and blood vessels structures and functions. These complications represent major cause of morbidity and mortality associated with diabetes [2]. One of the major pathways involved in the development and progression of different diabetic complications is protein glycation reaction leading to the formation of advanced glycation end products [3]. Some of the diabetic complications associated with this process include but not limited to nephropathy, retinopathy and neuropathy [4]. Other consequences of advanced glycation end products (AGEs) include cardiovascular complications, neurodegenerative derangement, bone disorders and erectile dysfunction to mention but a few [5].

* Corresponding author: Mbagwu Ikechukwu Sonne.

Advanced glycation end-products are the final products of the non-enzymatic reaction between reducing sugar and amino groups in proteins, lipoproteins and nucleic acids [6]. During sustained hyperglycemia, glucose creates covalent adducts with amino acid residues in proteins, which initiates this process. This process of protein glycation induces conformational changes that affects the structural and functional activities of proteins including their ability to act as receptors for ligand/drug interaction as well as their metabolic functions [7].

The pathological implications of AGEs are ascribed to their ability to produce reactive oxygen and nitrogen species as well as oxidative stress and inflammatory conditions that lead to structural and functional protein alteration, cellular dysfunction and other tissue and organ damages [8]. The free radicals generated through various glycation steps and other hyperglycaemic mediated processes also produce lipid peroxidation with its associated reactive carbonyl products [9]. The lipid peroxidation reactive products act as both precursor of AGEs formation and as direct protein damage initiator thereby amplifying the hyperglycaemia mediated protein damage [5]. The inhibition of advanced glycation end products ad lipid peroxidation therefore represents an important pharmacological target for the prevention or mitigation of life threatening complications of diabetes.

For many decades, natural products have been used as a source of therapeutic agents because of the existence of various bioactive compounds and they are believed to be non-toxic to a larger extent [10]. *Newbouldia laevis* commonly referred to as the African Border Tree or Boundary Tree can be seen growing in tropical rain-forest regions and has been used for many years in the practice of traditional medicine. This plant is also rich in many phytochemical constituents and secondary metabolites including Alkaloids, Flavonoids, Saponins, Tannins, Glycosides Phenols and the vitamins A, B1, B2, B6, B12, C, D, E and K which are ethno-pharmacologically significant [11]. *N. laevis* also exhibit anti-inflammation, antihypertensive, anticoagulant, antibacterial, and antioxidant properties which are comprehensively reported in the current literature [12]. *Newbouldia laevis* has been shown in recent research to have antidiabetic potential, with hypoglycemia effects similar to those of glibenclamide [13, 14]. In type 2 diabetes animals, the butanol fraction improved insulin secretion, lipid profile, and glucose/fat tolerance while significantly lowering blood glucose, body weight, and insulin resistance [15]. Additionally, the plant has also been reported to exert it's antidiabetic effects by lowering oxidative stress and decreasing the absorption of glucose, which affects insulin sensitivity and pancreatic β-cell activity [16]. In addition, Newboulasides A and B, two novel caffeic acid glycosides discovered from *N. laevis*, demonstrated potent α-amylase inhibition [17], indicating that enzyme inhibition is a crucial mechanism. These findings support its long-standing use in the treatment of diabetes. This study seeks to investigate further therapeutic potentials of *N. laevis* in the prevention and management of diabetes complications.

2. Materials and Methods

2.1. Plant material

Newbouldia laevis leaves were collected in Oba, Enugu State, Nigeria, and confirmed by a trained taxonomist, Mr. Felix Nwafor from the Department of Pharmacognosy and Environmental Medcine, University of Nigeria Nsukka, Enugu State, Nigeria. The plant material was air dried and crushed to powder. The voucher specimen was placed in the herbarium of the Faculty of Pharmaceutical Science at Nnamdi Azikiwe University's Agulu Campus (PCG 474/A/035).

2.2. Extraction

A 2.5 kg quantity of pulverized leaves of *N. laevis* was cold macerated in 10 L of aqueous ethanol (70%) for 72 h with intermittent shaking. The resulting solution was filtered, and the filtrate was pre-concentrated *in vacuo* using a rotary evaporator at 40°C and thereafter, dried to a constant weight using an open water bath at the same 40°C to obtain the ethanol extract.

2.3. Fractionation (Liquid-liquid Chromatography)

The ethanol extract (112 g) was dissolved in distilled water and subjected to liquid–liquid partition successively with 2.5 L of n-hexane, ethyl acetate, and then butanol using separating funnel to obtain the n-hexane, ethyl acetate and butanol soluble fractions, respectively. The leftover portion after partitioning was used as the water fraction. The fractions were pre-concentrated using rotary evaporator at 40^{o}C and dried using water bath at 40^{o}C. The water fraction was freeze dried.

2.4. *In vitro* analysis

2.4.1. Total phenolic content of the extract and fractions by Folin ciocalteu's assay

The total phenolic content of the fractions were determined using the method described by Kim *et al*. [18]. One milliliter of the extracts (100 µg/ml) was mixed with 0.2 ml of Folin-Ciocalteu's phenol reagent. After 5 minutes, 1 ml of 7.6% $N_{a2}CO_3$ solution was added to the mixture followed by the addition of 2 ml of distilled water. The mixture (in duplicate) was incubated at 40 °C for 30 minutes, after which the absorbance were read at 760 nm using UV-VIS spectrophotometer against blank (containing every other component of the mixture except sample). The total phenolic content was estimated from the calibrated curve which was made by preparing gallic acid solution and expressed as milligrams of gallic acid equivalent (GAE) per gram of the extracts.

2.4.2. Ferric reducing antioxidant power assay

The FRAP assay was conducted according to the method reported by Benzie and Strain, [19]. FRAP reagent was freshly made by mixing three solutions a, b and c, 300 mM sodium acetate buffer, pH = 3.6 (solution a), 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl solution (b) and 20 mM ferric chloride (FeCl3) solution (c) in proportions of 10:1:1 (v/v/v). The reaction was completed by keeping the reagent in darkness for 30 minutes. In the test, 0.1 mL of fractions and positive control ascorbic acid (100, 200, 400, 800, and 1600 µg/mL) and FeSO4 (200, 400, 600, 800, and 1000 µM) were mixed with 2.9 mL of FRAP reagent individually. An equal amount of 5% Tween 80 (0.1 mL) was used as a blank (control). All samples were prepared in triplicate and vortexed for 1 minute before being incubated in the dark at 37 °C for 30 minutes. Each sample's rise in absorbance was evaluated using a UV-Visible spectrophotometer at 593 nm. The results were compared with ascorbic acid as positive control and FeSO4 was used for calibration. FRAP activity was estimated using ferrous equivalent (FE) in µM.

2.4.3. Linoleic acid peroxidation assay

The procedure was performed according to modified method of Choi et al. [20]. The fractions were mixed with 550 µL linoleic acid solution (0.28 mg linoleic acid and 0.28 mg Tween-20 in 100 µM phosphate buffer,pH 7.4) at various concentrations (100 – 1600 µg/mL), 500 µL of phosphate buffer (100 µM, pH 7.4) and 150 µl of ascorbic acid (10 µM). Same concentrations of Trolox where used as reference standard while the blank contained the vehicle (5% Tween 80) in place of sample/standard. The linoleic acid peroxidation was initiated by the addition of 0.1 mL FeSO4 (10 µM) and incubated at 37°C for 60 min. The reaction mixture was cooled and 1.5 mL of trichloroacetic acid (10% in 0.5% HCl) added. Then, 3 mL TBA (1%, in 50 mM NaOH) was added. The reaction mixture and TBA solution were heated in the water bath at 90oC for 60 min. After cooling, 2 mL portions were taken from each sample and vortexed with 2 mL butanol and centrifuged at 1000 x g for 30 min. The upper layer of the resulting solution was separated for spectrophotometric measurement. The absorbance of solution was read at 532 nm and calculated the percentage of linoleic acid peroxidation inhibition in the following equation:

Linoleic acid peroxidation inhibition (%) = ((Acontrol-Asample)/ Acontrol) x100

2.4.4. In vitro glycation of bovine serum albumin

The glycated BSA formation was determined according to a previously described method (Adisakwattana *et al*. [21] with slight modification. Bovine serum albumin (BSA) was used as the model protein in a concentration of 40 mg/ml corresponding to physiological albumin concentration in human blood; similarly, glucose (0.5 M) was used as the glycating agent. Briefly, BSA (40 mg/ml) was incubated with 0.5 M glucose in 0.1 M phosphate buffer saline (PBS) (pH 7.4), containing 0.02 % sodium azide in the dark at 37 °C for 1, 2, 3, and 4 weeks. The solution containing *N. laevis fractions* (0.1–1.6 mg/ml) dissolved in PBS was added to the mixtures, before incubation. All incubations were performed under sterile conditions. A small drop of chloroform was added to the solution and the corks moistened with toluene to inhibit bacterial growth. Fluorescent intensity at an excitation wavelength of 355 nm and an emission wavelength of 460 nm was used to measure glycated BSA formation. Aminoguanidine (AG, 1 mg) was used as a positive control for the study.

$$\text{AGE inhibition (\%)} = \left[1 - \frac{Fs - Fsb}{Fc - Fcb}\right] x\ 100$$

Where Fs – Fsb is the difference between the fluorescent intensity of sample + BSA incubated with or without glucose while Fc - Fcb is the difference between the fluorescent intensity of BSA incubated with or without glucose.

2.4.5. Determination of protein carbonyl content

Carbonyl content is regarded a glycation indicator and is utilized as a measure to evaluate protein oxidation as well as a confirmatory assay for AGE production. The level of carbonyl group in glycated BSA was slightly modified according to Levine's method [22]. Concisely, 100 µl of glycated BSA was mixed with 400 µl of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2.5 M HCl. After incubation for 60 min at room temperature, glycated BSA was then precipitated using 500 µl of 20 % (w/v) trichloroacetic acid (TCA), left on ice for 5 min, and centrifuged at 10,000 g for 10 min at 4 °C. The protein pellet was washed 3 times by 500 µl of 1:1 (v/v) ethanol: ethyl acetate solution. The final protein pellet was resuspended in 250 µL of 6 M guanidine hydrochloride. The absorbance was read at 370 nm. The protein carbonyl group of each sample was calculated by using absorption coefficient (ε=22,000 M−1.cm−1). The protein carbonyl content was expressed as nmol carbonyl/mg protein.

2.5. Statistical analysis

Data were expressed as means ± standard error of mean (S.E.M), N=3. Data were analyzed using One way ANOVA followed by Tukey's HSD post hoc test. P-value

3. Results

3.1. Total phenolic content, ferric reducing antioxidant power and lipid peroxidation

The intermediary polar solvent (ethyl acetate) partitioned majority of the phenolic compounds making it the phenol rich fraction (Table 1). Lowest content was found in the non-polar solvent fraction (n-hexane) while water fraction contained about double fold lower the quantity recorded in ethyl acetate fraction.

The fractions showed graded concentration dependent reduction of ferric ion to ferrous ion (Figure 1). Using regression equation generated from the ferrous calibration curve (Y = 0.0009x – 0.1324), the fractions showed similar trend of effect based on their phenolic content. Ethyl acetate fraction showed the strongest ferric reducing power while n-hexane fraction the least. These reducing powers shown by the fractions were however lower when compared to corresponding concentrations of the reference standard (ascorbic acid).

For the inhibition of lipid peroxidation, n-hexane fraction produced linear concentration-effect curve compared to other fractions that produced hyperbolic curve shape just like the reference standard (trolox) (Figure 2). The order of inhibition of lipid peroxidation by the fractions followed the same trend with phenolic content. Ethyl acetate fraction inhibited 50% of lipid peroxidation at 0.349 mg/ml which is the closest compared to trolox - the reference standard (0.15 mg/ml). n-hexane required upto 6768 mg/ml concentration to produce same inhibition of 50% lipid peroxidation.

Table 1 Total phenolic content of the fractions of *N. laevis* extract

Fractions of *N. laevis* Extract	**Total Phenolic content (mgGAE/g)**
N-hexane	46.87 ± 1.10
Ethyl acetate	389.91 ± 4.74
Butanol	236.85 ± 2.56
Water	107.79 ± 4.02

Where GAE = Gallic acid equivalent

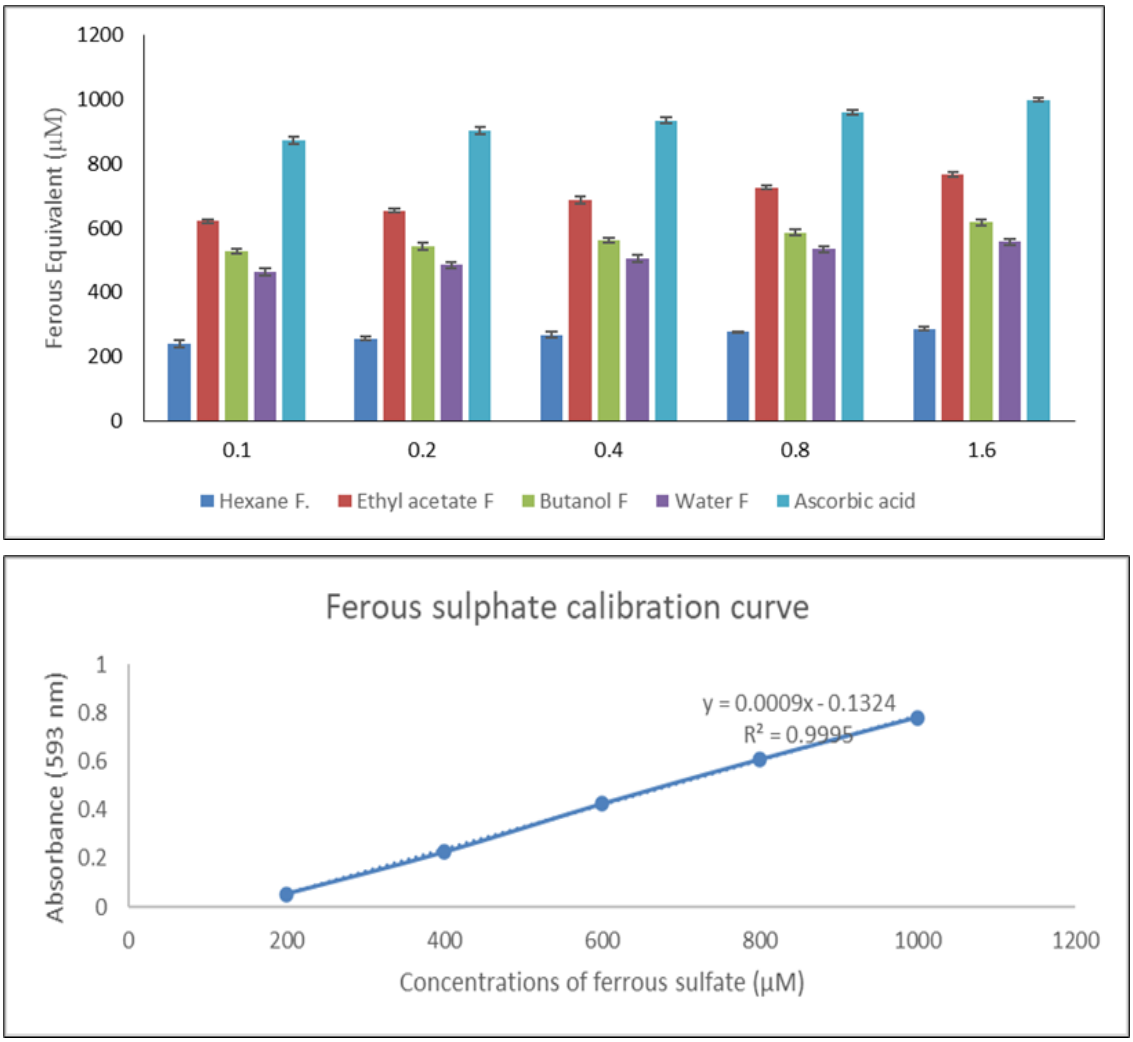
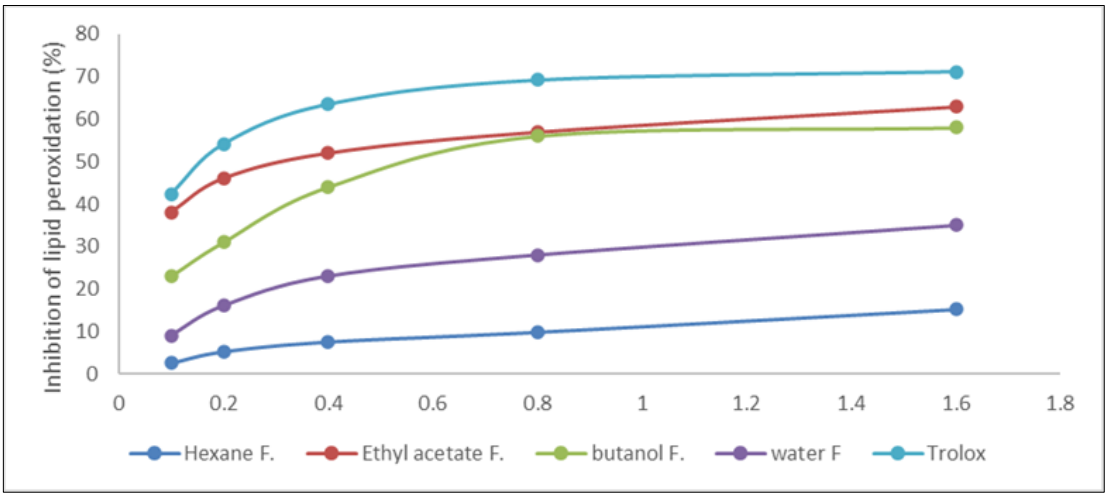


Figure 1 Ferric reducing antioxidant power effect of the fraction



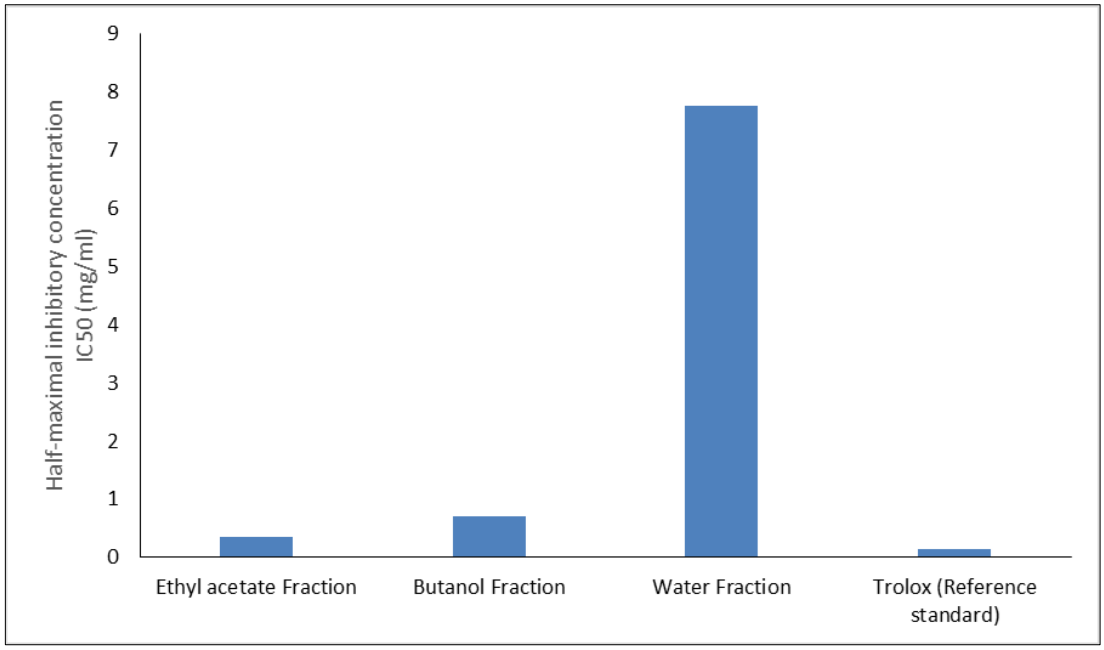


Figure 2 Inhibitory effect of the fraction on lipid peroxidation

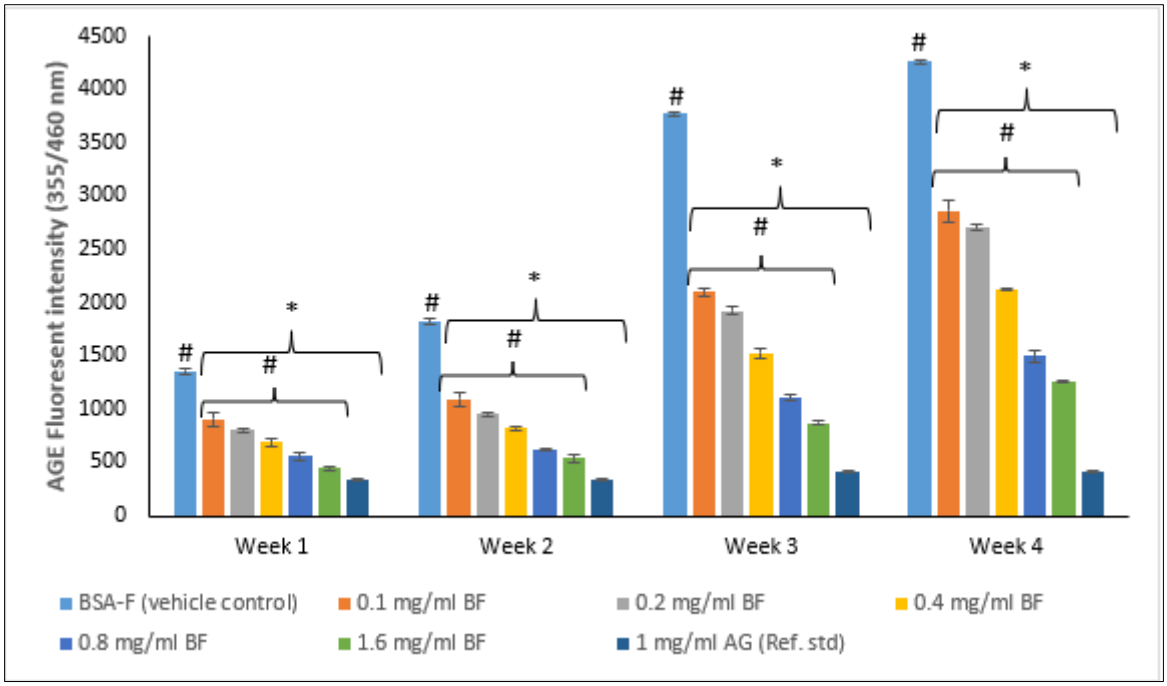
3.2. Effect of treatment on advanced glycation end (AGE) product formation

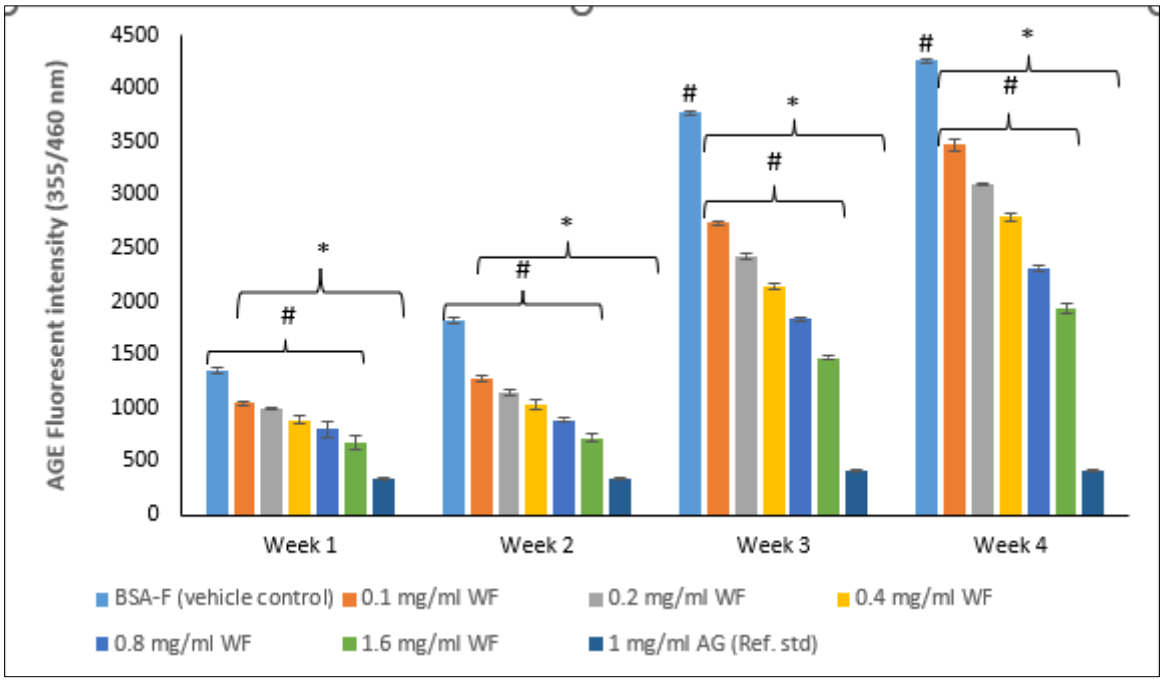
The formation of AGE in bovine serum albumin (BSA) was measured by the fluorescence intensity. As shown in figure 3, the fluorescent intensity increased with time during 4 weeks of experiment in the vehicle control group (BSA-F).

The butanol and water fractions showed concentration dependent inhibition of AGE formation. The fluorescent intensity recorded by the graded concentrations of the fractions were significantly ($P<0.05$) lower than that of the vehicle control at all points of measurement (figure 3). The effect recorded by these fractions were significantly ($P<0.05$) lower than that shown by the reference standard (Aminoguanidine).

The n-hexane fraction showed lower ability to inhibit AGE formation compared to other fractions. Significant ($P<0.05$) inhibition of AGE formation where shown in week 1 at the initiation of AGE formation (figure 4). With time, higher concentrations of n-hexane fraction were required to produce significant ($P<0.05$) inhibition of AGE formation. Ethyl acetate fraction recorded the highest effect of inhibition of AGE formation compared to other fractions as shown by significantly ($P<0.05$) lower fluorescent intensity compared to the vehicle control. At week 1, all the tested concentrations except 0.1 mg/kg showed similar strength of inhibition of AGE formation compared to the reference standard with no significant ($P>0.05$) difference when these concentrations were compared between groups. In weeks 2 - 4, 0.4 – 1.6 mg/ml concentrations of ethyl acetate fraction produced similar effect when compared to reference standard.

The inhibition of AGE produced by the fractions followed the same trend in all the weeks of the experiment with ethyl acetate fraction having the highest inhibition while n-hexane the least (figure 5). The half-maximal inhibitory concentration of ethyl acetate fraction at the end of the experiment (week 4) was 0.058 mg/ml while that of n-hexane was 1575 mg/ml. Similarly, butanol and water fractions required higher concentrations to inhibit 50% of AGE formation compared to ethyl acetate fraction (figure 6). However, these polar fractions (butanol and water fractions) produced better than the polar fraction (n-hexane fraction).





Where: * = P<0.05 compared to BSA-F (vehicle control); # = P<0.05 compared to Aminoguanidine (standard reference drug). Where BF = butanol fraction and WF = water fraction

Figure 3 Effect of butanol and water fractions on Advanced glycation end product formation

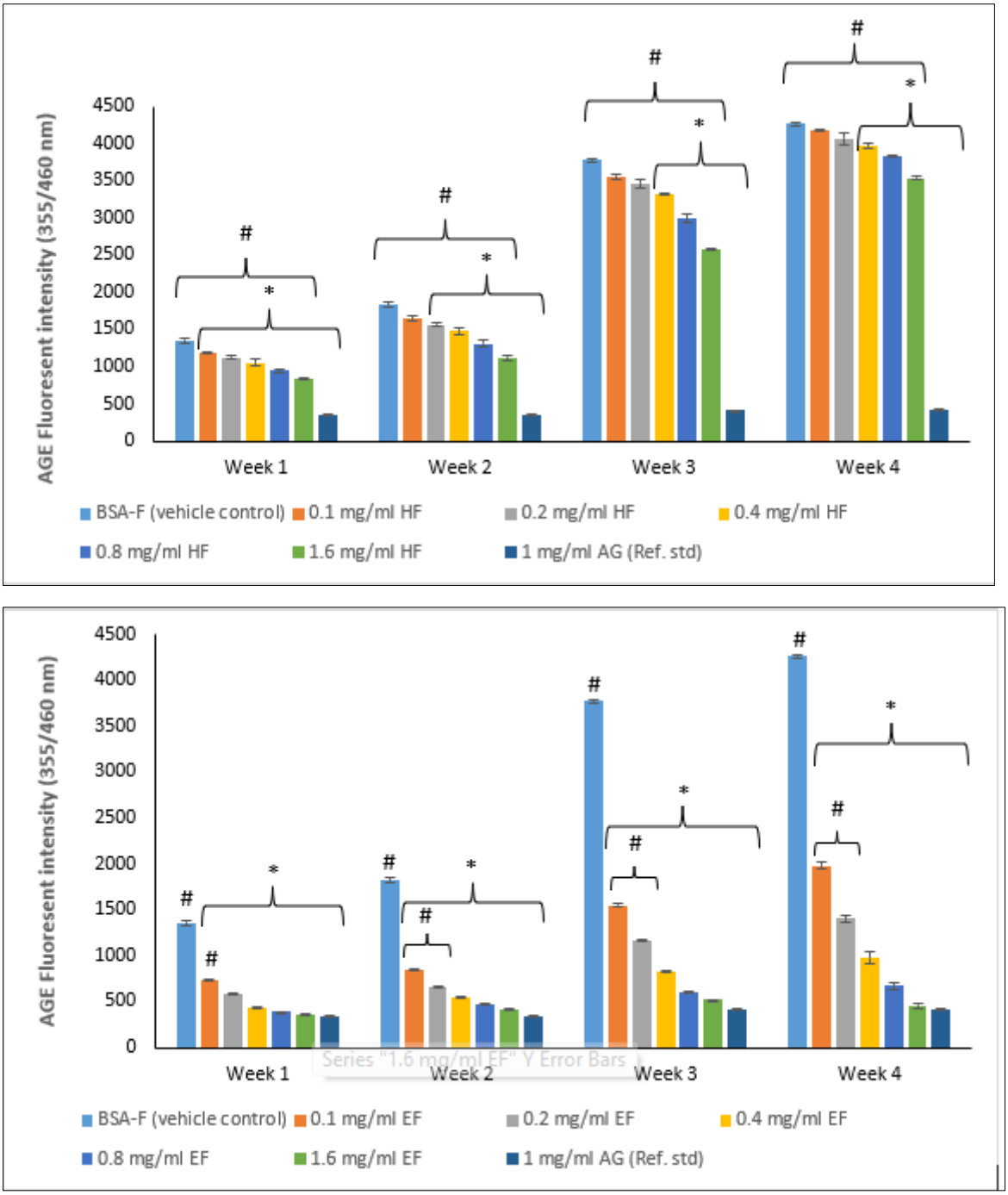


Figure 4 Effect of Hexane and Ethyl acetate fractions on Advanced glycation end product formation

Where: * = P<0.05 compared to BSA-F (vehicle control); # = P<0.05 compared to Aminoguanidine (standard reference drug). Where HF = hexane fraction and EF = Ethyl acetate fraction

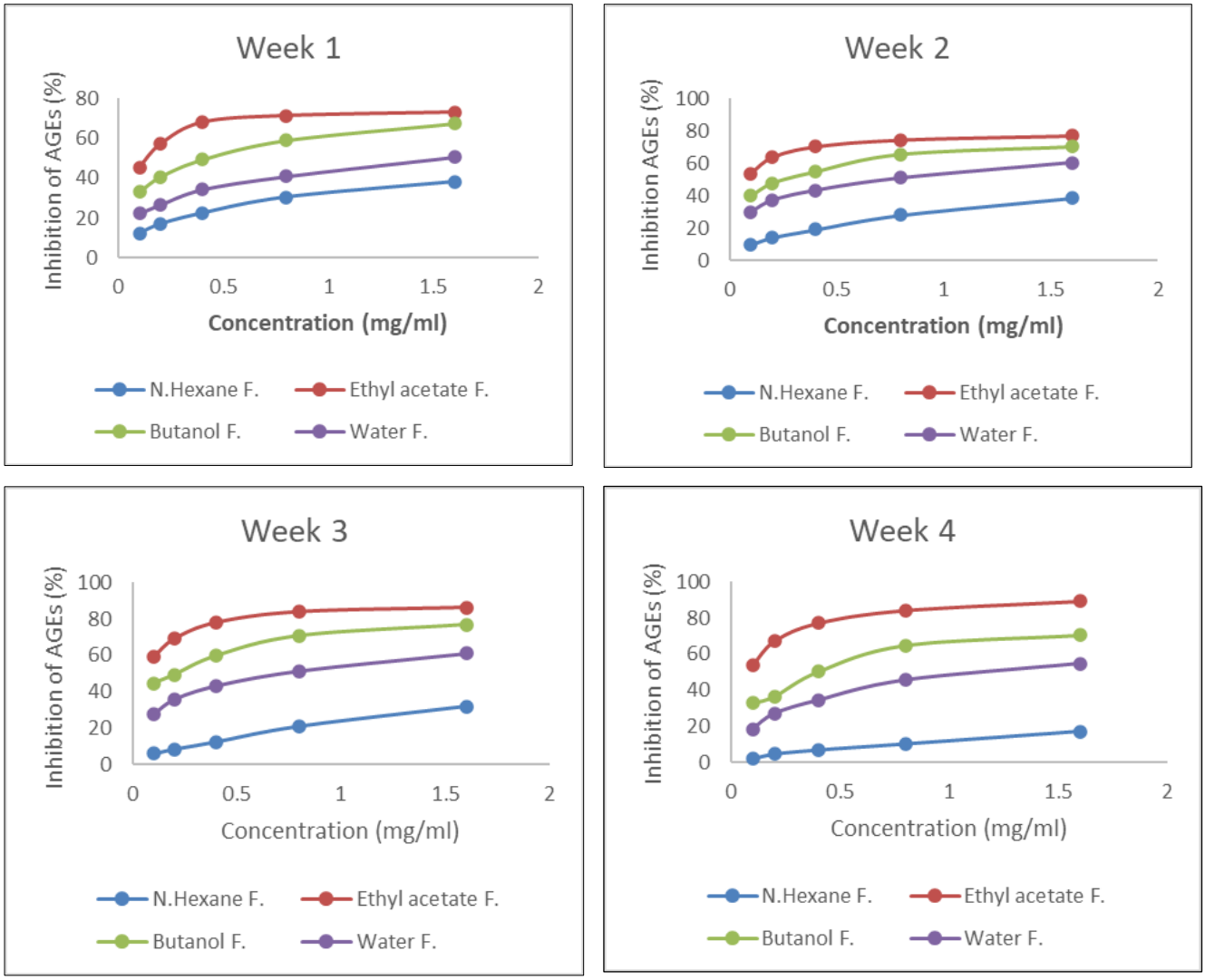
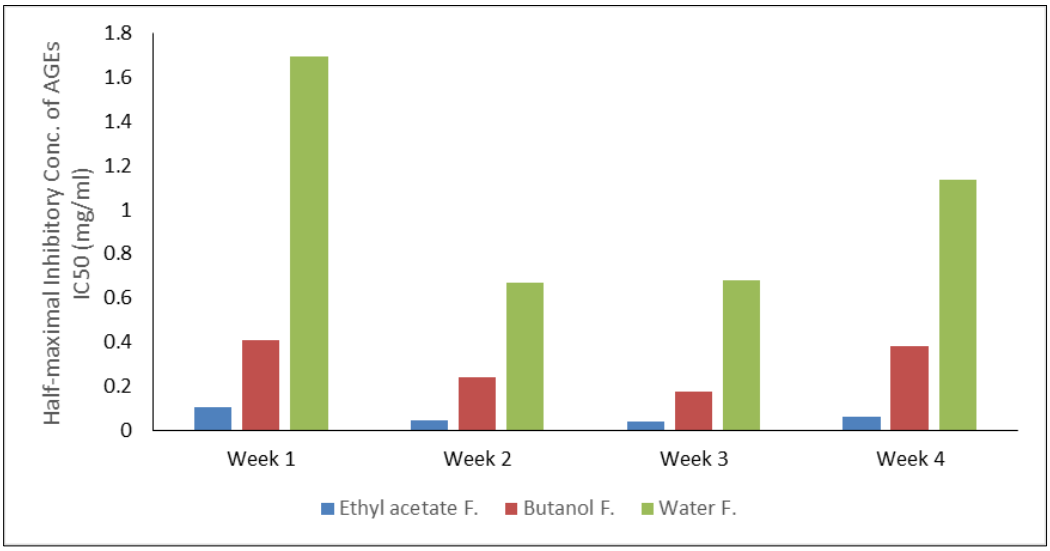


Figure 5 Graded concentration effect of solvent fractions of *N. laevis* extract on inhibition of Advanced glycation end product formation



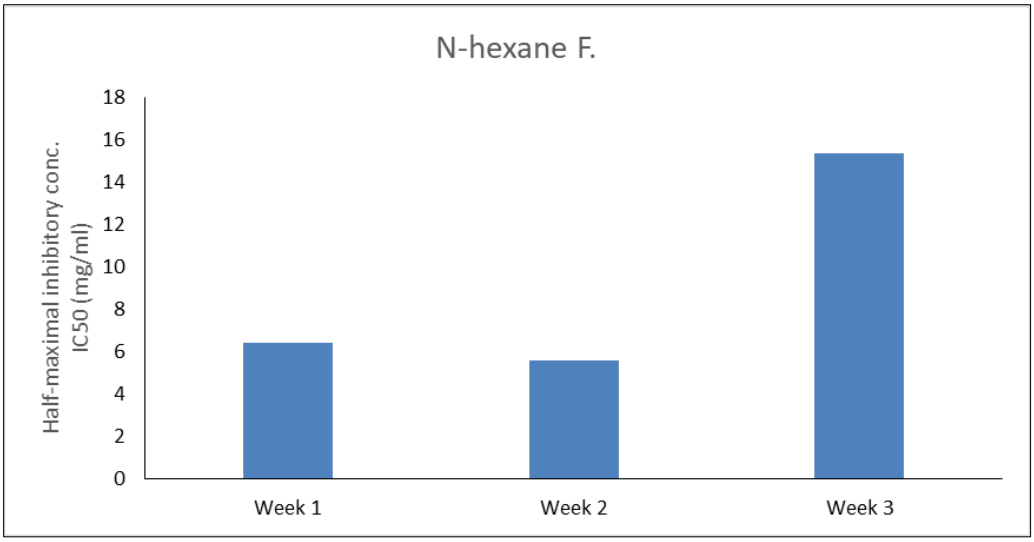


Figure 6 Half-maximal inhibitory concentrations of solvent fractions of *N. laevis* extract against Advanced glycation end product formation

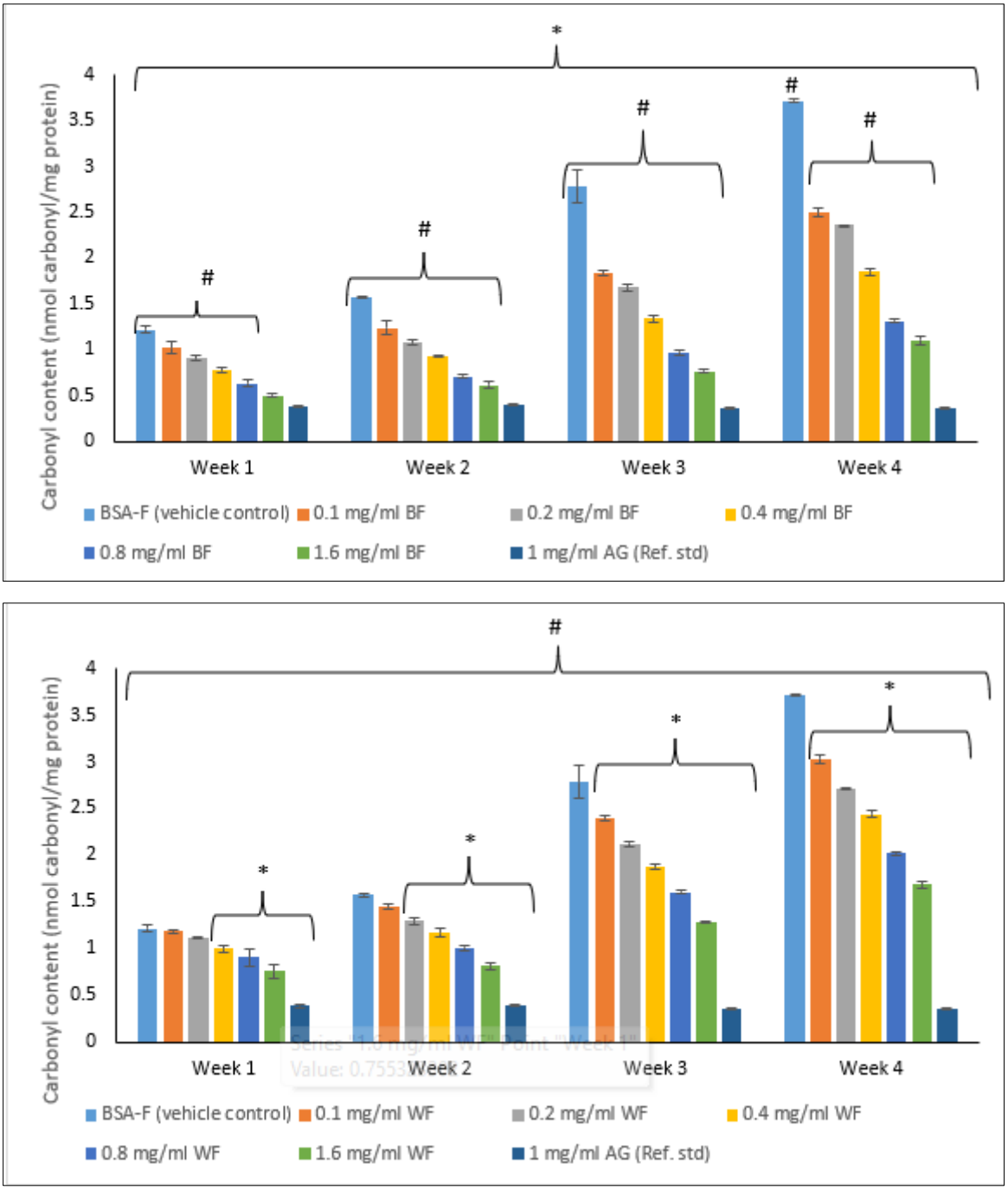
3.3. Effect of the fractions on glycation-induced protein oxidation

Glycation-induced protein oxidation was measured by carbonyl content. The carbonyl content of glycated BSA increased progressively from week 1 – 4. Addition of the fractions of *N. laevis* inhibited protein oxidation as shown by significant ($P<0.05$) reduction in carbonyl content compared to the vehicle control (BSA-F). Butanol fraction at all tested concentrations (0.1-1.6 mg/ml) produced significant ($P<0.05$) reduction in carbonyl content compared to the vehicle control (figure 7) while water fraction was able to achieve this at weeks 3 and 4. Lower concentrations of water fraction was unable to produce significant ($P<0.05$) reduction in carbonyl content in weeks 1 and 2. The effects recorded by 1 mg/ml of the reference drug (aminoguanidine) were significantly ($P<0.05$) higher than that produced by butanol and water fractions at all weeks.

Week inhibition of protein oxidation was shown by n-hexane fraction compared to other fractions (figure 8). Higher carbonyl content were recorded compared to vehicle control at lower concentrations of this fraction. In weeks 2 and 4, the carbonyl content produced by 0.1 – 0.4 mg/ml concentration of n-hexane fraction were significantly ($P<0.05$) higher than that produced by the vehicle control. Unlike the n-hexane fraction, the ethyl acetate fraction produced significant ($P<0.05$) reduction in carbonyl content at all tested concentrations compared to the vehicle control. Higher concentrations of ethyl acetate fraction (0.4 – 1.6 mg/ml) produced similar effect compared to the reference standard with no significant ($P>0.05$) difference when compared statistically.

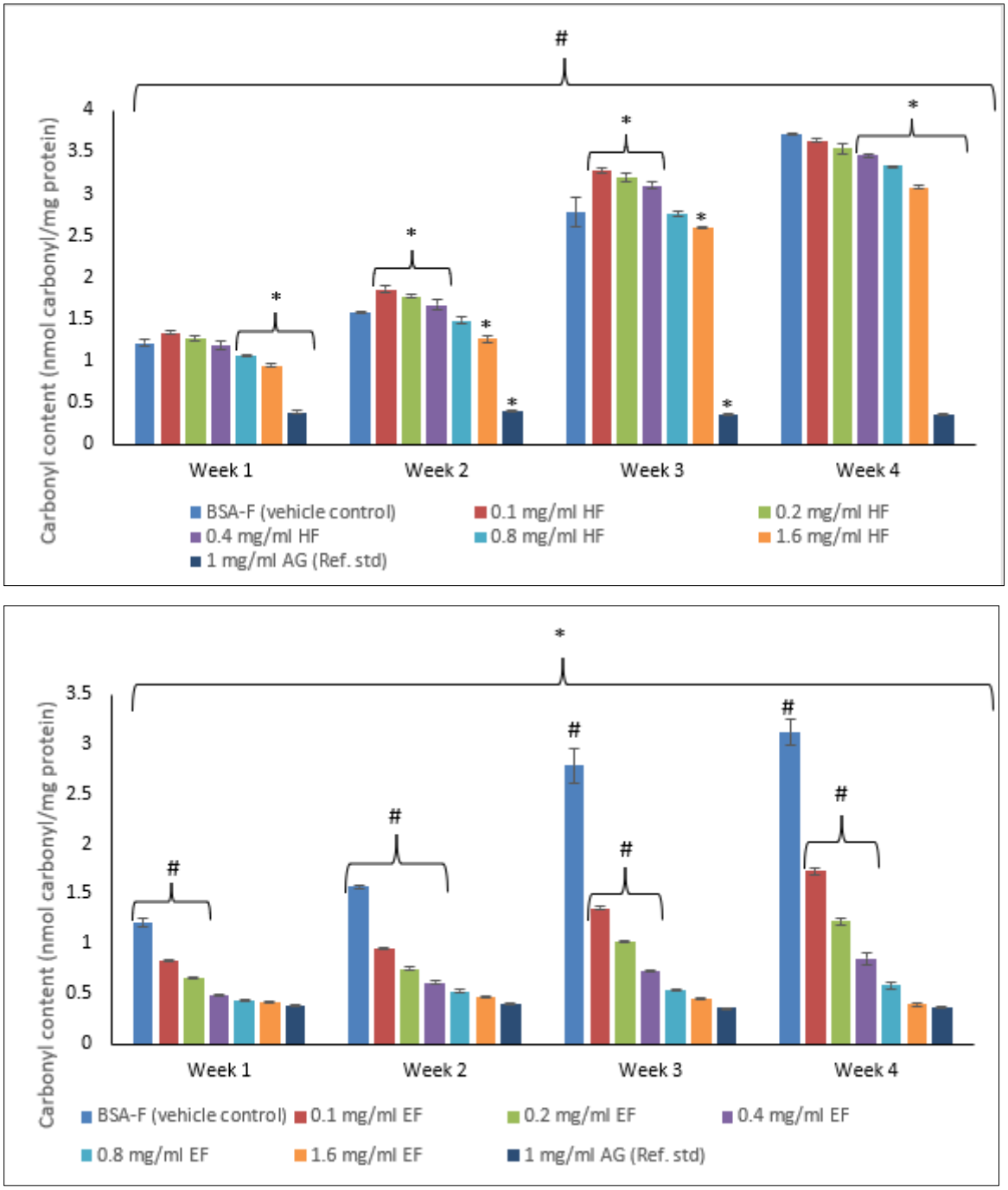
The graded concentration inhibitory curve of all the fractions showed same trend as in inhibition of AGE formation (figure 9). The ethyl acetate fraction produced highest inhibitory effect while n-hexane the least. Negative inhibitions were also recorded by lower concentrations of n-hexane fraction in all the weeks.

The half-maximal inhibitory concentration required for ethyl acetate fraction to inhibit 50% of the carbonyl content formation was 0.073 in week 3 and 0.11 in week 4 (figure 10). Other fractions produced lower potency with n-hexane fraction having the least potency.



Where: * = P<0.05 compared to BSA-F (vehicle control); # = P<0.05 compared to Aminoguanidine (standard reference drug). Where BF = butanol fraction and WF = water fraction

Figure 7 Effect of butanol and water fractions on protein carbonyl content



Where: * = P<0.05 compared to BSA-F (vehicle control); # = P<0.05 compared to Aminoguanidine (standard reference drug). Where HF = n-hexane fraction and EF = ethyl acetate fraction

Figure 8 Effect of hexane and ethyl acetate fractions on protein carbonyl content

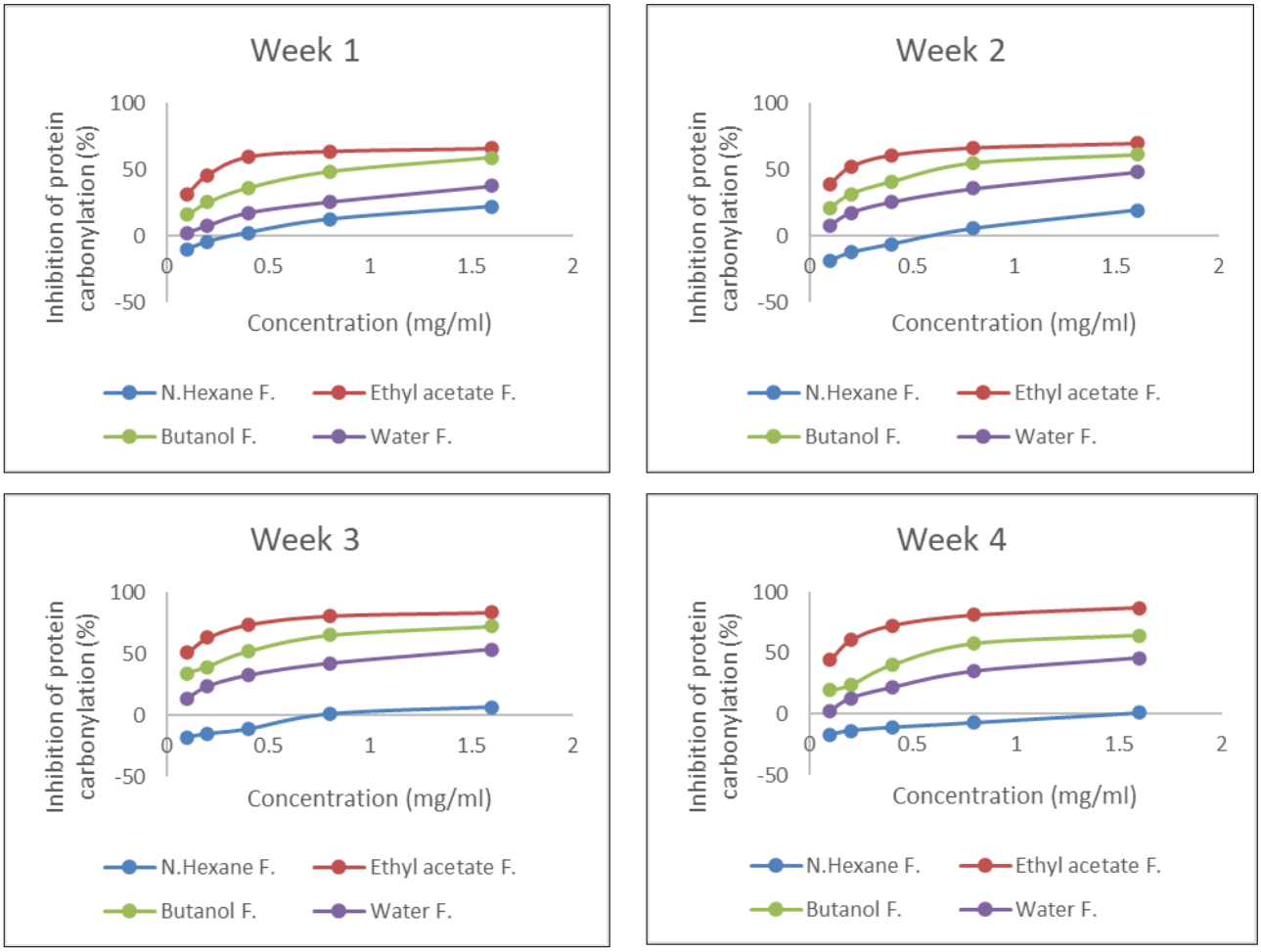
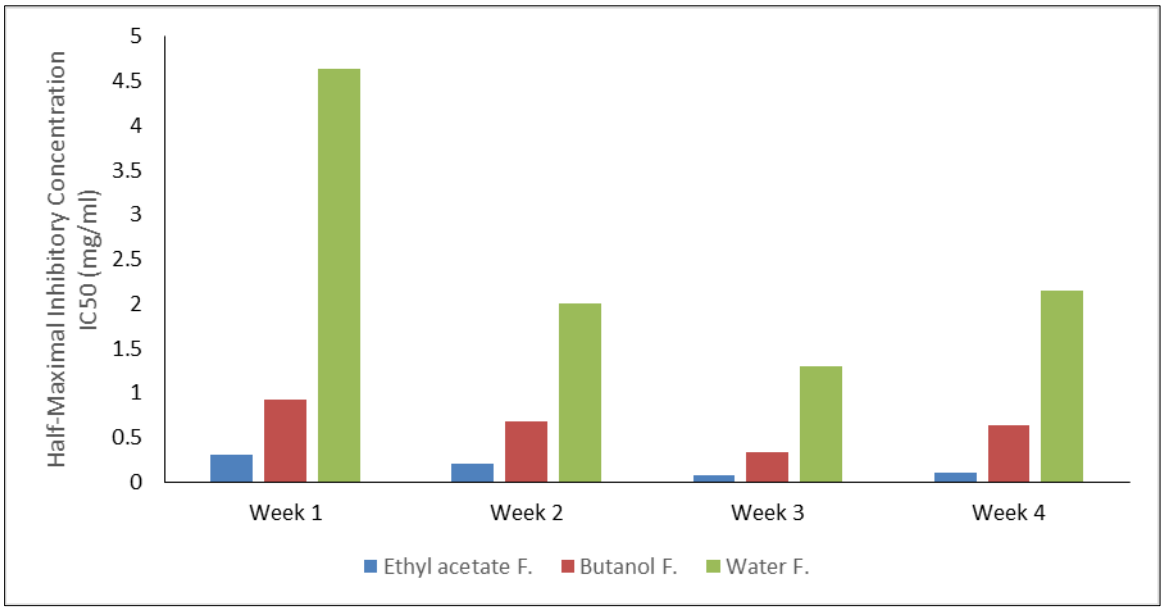


Figure 9 Graded concentration effect of solvent fractions of *N. laevis* extract on inhibition of protein carbonyl formation



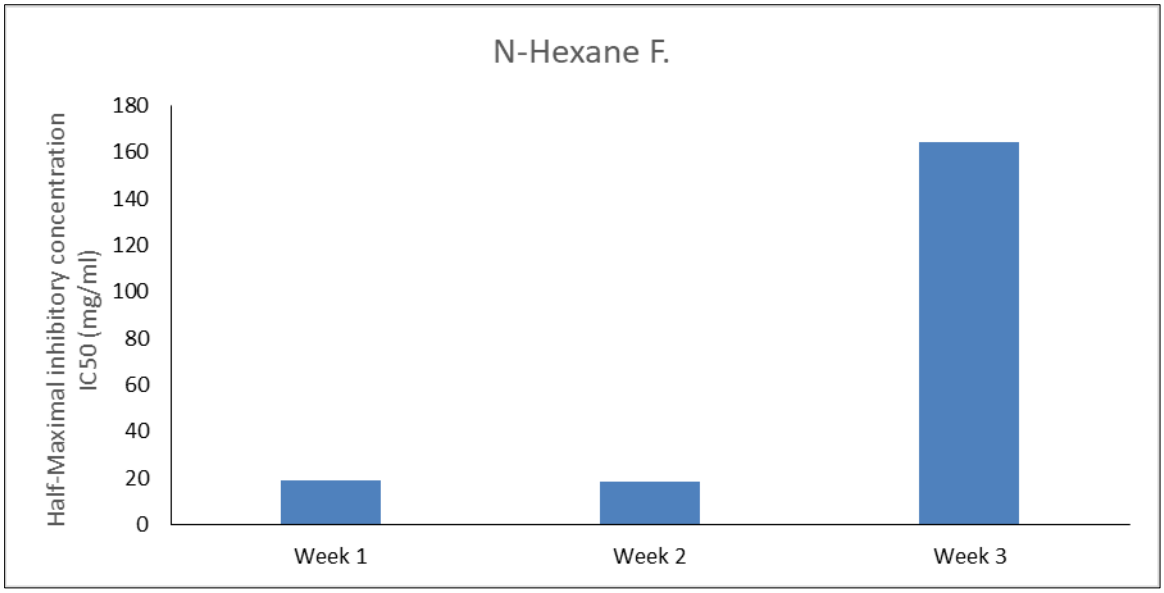


Figure 10 Half-maximal inhibitory concentrations of solvent fractions of *N. laevis* extract against protein carbonyl formation

4. Discussion

The induction of AGE formation and the subsequent modification of proteins play a significant role in the pathogenesis of various chronic diseases, especially those related to aging and metabolic disorders such as Alzheimers, eheumatoid arthritis, atherosclerosis and particularly diabetes with its associated complications [23].

In the early stage of glycation, the carbonyl group from reducing sugars (like glucose and fructose) reacts non-enzymatically with free amino groups in proteins to form freely reversible schiff's bases which are subsequently rearranged into a more stable Amadori products (eg HbA1c in diabetes and fructosamine). The Amadori product then undergoes further modifications like oxidation, dehydration and fragmentation generating superoxide and hydroxyl radicals as well as reactive carbonyl species such as glyoxal, methylglyoxal and 3-deoxyglucosone. These damaging reactive intermediates then react with proteins to produce AGEs [6]. Based on the fluorescence property of AGEs, The carbonyl group in glycated BSA was slightly changed according to Levine's method. In addition, the reduction of protein carbonyl content was effected by *N. laevis*. These activities as observed in the extract and fractions were consistent with the phenolic quantities in these samples

Antiglycation mechanisms involve either blockade of glycation at early stages by controlling excess reducing sugars like glucose from circulation, neutralization of reactive intermediates that react with proteins to form AGE or breakdown of existing AGEs. Phenolic compounds play a significant role in antiglycation mechanisms due to their strong antioxidant and carbonyl-scavenging properties [6]. By their structural features of electron donors, they neutralize reactive oxygen species (ROS) which promote oxidative steps in glycation. Through this mechanism, they produce inhibition of glycoxidation (oxidation of Amadori products) which leads to AGE formation. Phenolic compounds can directly react with reactive carbonyl compounds, neutralizing them before they modify proteins. Through this mechanism, they prevent formation of AGEs from dicarbonyl precursors [24]. *N. laevis* showed exhibited high phenolic content and antioxidant activity. Since their inhibition of AGEs formation and reactive carbonyl compounds are in the order of their phenolic distribution in the extract and different solvent fractions of the extract, these property may have contributed in their exhibited activity against AGEs formation. By reducing concentration of reactive carbonyl compounds, it is expected to translate to fewer AGEs formation since these reactive intermediates act as precursors to formation of AGEs.

Previous studies have reported the hypoglycermic effect of *N. laevis* in type 2 diabetic animal model and its inhibitory effect on alpha amylase enzyme as well as enhanced postprandial glucose tolerance. Its ability to improve insulin sensitivity in high fat diet diabesity mice model has also been reported [15, 25, 26]. These activities may have contributed in blockade of glycation at early stages through control serum glucose concentration which a reducing sugar that act as primary precursor in the reaction cascades of AGEs formation.

Caffeic acid a naturally occurring phenolic compound have been reported to inhibit reactive carbonyl compounds as well as chelate transition metals which catalyze oxidative reactions in glycation and may through these mechanisms reduce formation of ROS and glycoxidative products. By preventing carbonyl stress and oxidative damage, caffeic acid have been reported to preserve the native structure and function of proteins and through this mechanism maintain enzyme activity, receptor functionality and structural protein integrity which are usually adversely affected by AGEs [27]. Apart from direct reactive effects of AGEs, these products can also bind to their cell-surface receptor (receptor for advanced glycation end product – RAGE) triggering a pro-inflammatory and pro-oxidant signalling cascade which increases oxidative stress and amplifies inflammation. Caffeic acid reported activities extends to inhibition of RAGE gene expression at transcriptional level through blocking of ROS-mediated signalling that upregulates RAGE as well as suppression of transcription factors like NF-kb and AP-1 that enhance RAGE expression [28]. In our previous study, we identified and isolation caffeic acid compounds from *N. laevis* as bioactive compound responsible for its antidiabetic activity [17]. This provides insight that this same compound may be responsible for inhibition of AGEs formation, which will be a useful contribution to its known antidiabetic activity and a potential in combating complications of diabetes resulting from AGEs formation.

5. Conclusion

The extract and fractions of *N. laevis* showed inhibition of AGEs formation and lipid peroxidation. The degree of these activities was dependent on the total phenolic contents of the extract and fraction. Inhibition of AGEs formation and lipid peroxidation by *N. laevis* may therefore have been mediated by its phenolic phytocompounds

Compliance with ethical standards

Acknowledgments

The authors wish thank the management of Nnamdi Azikiwe University for providing laboratory facilities and technical support during the course of this study.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

Conceptualization, and M.I.S.; Methodology, D.L.A.; Software, A.A.A..; Validation, M.S.I., D.L.A. and A.A.A..; Formal Analysis, D.L.A.; Investigation, D. L. A.; Resources, M.S.I.; Data Curation, D.L.A.; Writing – Original Draft Preparation, D. L.A.; Writing – Review & Editing, A.A.A.; Visualization, A.A.A.; Supervision, M.S.I.; Project Administration, A.A.A.; Funding Acquisition, M.S.I.

Funding

This research received no specific grant from any funding agency (the public, commercial, or not-for-profit sectors).

References

[1] Khardor R. (2024). Type 2 Diabetes Mellitus. https://emedicine.medscape.com/article/117853-overview. (Accessed on 18/04/2025)

[2] Banday MZ, Sameer, A. S. and Nissar, S. (2020). Pathophysiology of diabetes: An overview. Avicenna J Med. 10(4), 174-188. doi: 10.4103/ajm.ajm_53_20.

[3] Khalid M., Petroianu G., Adem A. Advanced Glycation End Products and Diabetes Mellitus: Mechanisms and Perspectives. Biomol. 2022. 12(4): 542. doi: 10.3390/biom12040542.

[4] Li Y., Liu Y., Liu S., Gao M., Wang W., Chen Y. Diabetic vascular diseases: molecular mechanisms and therapeutic strategies. Signal Transd Target Ther. 2023. 8: 152. https://doi.org/10.1038/s41392-023-01400-z

[5] Chaudhuri J., Bains Y., Guham S., Kahn A., Hall D., Bose N. The Role of Advanced Glycation End Products in Aging and Metabolic Diseases: Bridging Association and Causality. Cell Metab. 2018. 28(3): 337-352. doi: 10.1016/j.cmet.2018.08.014.

[6] Uceda A.B., Mariño L., Casasnovas R., Adrover M. An overview on glycation: molecular mechanisms, impact on proteins, pathogenesis, and inhibition. Biophys Rev, 2024. 16: 189–218. https://doi.org/10.1007/s12551-024-01188-4

[7] Singh V.P., Bali A., Singh N., Jaggi A.S. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2014. 18(1): 1-14. doi: 10.4196/kjpp.2014.18.1.1.

[8] Leyane T.S., Jere S.W., Houreld N.N. Oxidative Stress in Ageing and Chronic Degenerative Pathologies: Molecular Mechanisms Involved in Counteracting Oxidative Stress and Chronic Inflammation. Int J Mol Sci. 2022. 23(13): 7273. doi: 10.3390/ijms23137273.

[9] Chen Y., Meng Z., Li Y., Shibo L., Hu P., Luo E. Advanced glycation end products and reactive oxygen species: uncovering the potential role of ferroptosis in diabetic complications. Mol Med. 2024. 30: 141. https://doi.org/10.1186/s10020-024-00905-9.

[10] Nasim N., Sandeep I.S., Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus. 2022. 65(3): 399-411. doi: 10.1007/s13237-022-00405-3.

[11] Rashed K. Phytochemical and Biological Effects of Newbouldia laevis: A Review. Plantae Sci. 2021. 4(5): 208-213. DOI: 10.32439/ps.v4i5.208-213

[12] Nwozo S., Effiong M., Aja P.M., Awuchi C.G. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review· Int J Food Prop. 2023. 26(1): 359-388. DOI: 10.1080/10942912.2022.2157425

[13] Osigwe C.C., Akah P.A., Nworu C.S. Biochemical and Haematological Effects of the Leaf Extract of Newbouldia laevis in Alloxan-Induced Diabetic Rats. J Biosci. Med. 2017. 5(6): 18–36. doi:10.4236/jbm.2017.56003.

[14] Sédégo R., Bakouan Y., Ganamé H.T., Yoda J., Benjamin O., Sessouma B. Antiradical and Antidiabetic Potential of Newbouldia laevis (P. Beauv.) ex Bureau Leaf Extracts: Inhibition of α-Glucosidase Activity. J Chem. 2024. 14(5): 55-62. DOI: 10.5923/j.chemistry.20241405.02.

[15] Mbagwu I.S., Akah P.A., Ajaghaku D.L. Newbouldia laevis improved glucose and fat homeostasis in a TYPE-2 diabesity mice model. Journal of Ethnopharmacol. 2020. 251(6): 112555. https://doi.org/10.1016/j.jep.2019.112555

[16] Mbagwu I.S., Akah P.A., Ajaghaku A.A., Ugwu O.C., Ajaghaku D.L. Inhibition of oxidative stress and gastric emptying as additional mechanisms of antidiabetic activity of Newbouldia laevis. Phytomed Plus. 2021, 1(2): 100023. https://doi.org/10.1016/j.phyplu.2021.100023

[17] Mbagwu I.S., Akah P.A., Ajaghaku D.L., Ike J.C., Okoye F.B. Newboulasides A and B, two new caffeic acid glycosides from Newbouldia laevis with α-amylase inhibitory activity. Nat Prod Res. 2022. 36(3): 726-734. doi: 10.1080/14786419.2020.1799362.

[18] Kim D., Chun O., Kim Y., Moon H., Lee C. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem. 2003. 51: 6509–6515. https://doi.org/10.1021/jf0343074

[19] Benzie I.F., Strain J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidat power": the FRAP Assay. Anal. Biochem. 1996. 239(1): 70-76. https://doi.org/10.1006/abio.1996.0292

[20] Choi C.W., Kim S.C., Hwang S.S., Choi B.K., Ahn H.J., Lee M.Y. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guideid comparison. Plant Sci. 2002. 163: 1161–1168. https://doi.org/10.1016/S0168-9452(02)00332-1

[21] Adisakwattana S, Sompong W, Meeprom A, Ngamukote S, Yibchokanun S. Cinnamic acid and its derivatives inhibit fructosemediated protein glycation. Int J Mol Sci. 2012. 13: 1778–1789. https://doi.org/10.3390/ijms13021778MDPI

[22] Levine R.L. Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med. 2022. 32: 790–796. https://doi.org/10.1016/S0891-5849(02)00765-7

[23] Twarda-Clapa A., Olczak A., Białkowska A.M., Koziołkiewicz M. Advanced Glycation End-Products (AGEs): Formation, Chemistry, Classification, Receptors, and Diseases Related to AGEs. Cells. 2022. 11(8): 1312. doi: 10.3390/cells11081312.

[24] González P., Lozano P., Ros G., Solano F. Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. Int J Mol Sci. 2023. 24(11): 9352. doi: 10.3390/ijms24119352.

[25] Osigwe C.C., Akah P.A., Nworu S.I., Okoye T.C., Tchimene M.k. Antihyperglycemic Studies on the Leaf Extract and Active Fractions of Newbouldia laevis (Bignoniaceae). Pharmacol. Pharm. 2015. 6(11): DOI: 10.4236/pp.2015.611054

[26] Chellappan D.K., Chellian J., Rahmah N.S., Gan W.J., Banerjee P., Sanyal S., Palaniveloo K. Hypoglycaemic Molecules for the Management of Diabetes Mellitus from Marine Sources. Diabetes Metab Syndr Obes. 2023. 16: 2187-2223. doi: 10.2147/DMSO.S390741.

[27] Yadav N., Palkhede J.D., Kim S.Y. Anti-Glucotoxicity Effect of Phytoconstituents via Inhibiting MGO-AGEs Formation and Breaking MGO-AGEs. Int J Mol Sci. 2023. 24: 7672. https://doi.org/10.3390/ijms24087672.

[28] Vianello E., Beltrami A.P., Aleksova A., Janjusevic M., Fluca A.L., Corsi Romanelli M.M., La Sala L., Dozio E. The Advanced Glycation End-Products (AGE)–Receptor for AGE System (RAGE): An Inflammatory Pathway Linking Obesity and Cardiovascular Diseases. Int J Mol Sci. 2025. 26: 3707. https://doi.org/10.3390/ijms26083707.