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SPECTRAL STUDIES FOR THE DETERMINATION OF THE *IN VITRO* ANTI-DIABETIC AND ANTIOXIDANT PROPERTIES OF *AFRAMOMUM DANIELLI*

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Abstract

Analysis and researches carried out on the importance of medicinal plants are gaining relevance across the globe. This study determined the various constituents present in Aframommum danielli ethanol extract, the antioxidant and anti-diabetes efficacies in vitro. Phytochemical screening was carried out using standard analytical methods. Spectral studies were carried out through ultra violet (UV), and gas chromatography-mass spectrometry (GC-MS). Also in vitro studies of the antioxidant parameters like α, α-diphenyl-β-picrylhydrazyl (DPPH), nitric oxide, metal chelating, hydroxyl radical, radical scavenging assays, and anti-diabetes inhibitory α-amylase and α-glucosidase assays were determined. Result showed that ethanol extracts of A. danielli, is rich in flavonoids, (0.032±0.001), tannins (0.858±0.006) alkaloids (0.105±0.035), phytate (1.294±0.107), oxalates (2.769±0.095), cyangenic glycosides (45.977±0.109). A large number of peaks with good area percentage indicating functional groups were found with some compounds like 2-Butanone, 4-(4-hydroxy-3-methoxy phenyl), Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester, benzenesulfonamide, N-(4-amino-2-methoxyphenyl), 1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one, 4-Hydroxy-3-methoxybenzyl alcohol, di(2-methylpropyl) ether. The highest scavenging activity (58.45±0.92) was shown by DPPH ethanol extract of A. danielli. Moreover, the control scavenging ability of the plant extracts was in the order of NO>DPPH>OH^-> metal chelating with lowest antioxidant activity (20.27±0.17) when compared with the control, silymar*in. Good antioxidant action was examined in the extract. The extract showed highest α-amylase inhibitory activity (3.81±0.32) and α-glucosidase (49.93±1.98), when compared with the standard acarbose. It can be concluded that the extract of A. danielli is rich in bioactive compounds and can be adopted in the prevention of oxidative stress and treatment of diabetes.*

Keywords: *Aframommum danielli,* Diabetes, Oxidative stress, Antioxidant, Anti-diabetes

Introduction

*Aframomum danielli (*Alligator pepper) as commonly called is widely used by many cultures in Nigeria and across the globe for various purposes (Mclaughlin, Chang and Smith, 1991). It is a common ingredient in pepper soup, a spicy delight in most parts of West Africa and concoctions made of alligator peppers are often used by traditional doctors as medications for various ailments (Mclaughlin *et al*., 1991). It is a two sesquiterpene hydrocarbons, humelene and caryophyllene, their oxides and a few non-terpenoids (Ajaiyieoba and Ekundayo, 1999). *A. danielli* seeds are an excellent source of phytonutrients such as terpenoids, alkaloids, flavonoids, tannins, cardiac glycosides, saponin and phenolic compounds (Igwe *et al*., 1999). They scavenge for free radicals and offer protection against viruses, allergens, microbes, platelet aggregation, tumors, ulcers and hepato-toxins (can cause chemical liver damage) in the body (Jabeen *et al*., 2004).

Across the globe, diabetes mellitus (DM) is prevalent and on the increase with over 380 million people being affected (Guariguata *et al.,* 2014). This condition, hyperglycemia has been linked with increase in the generation of reactive oxygen species (ROS), which binds to carbohydrates, proteins, lipids or DNA thus altering the molecular structures, leading to oxidative stress and finally diseased state (Devasagayam *et al.,* 2007; Antonioni et al., 2019).

Some biological activities such as anti-diabetes, anti-microbial, antibacterial, anti-inflammatory and antioxidant activities have been reported for the *Aframomum* families (Ngane, 2013; Lawal *et al.,* 2015). However, the need for natural products that can function as antioxidants, anti-diabeteic, anti-inflammatory, most especially those of plant source has been on the increase of recent. This work was, therefore, carried out to determine the composition as well as the antidiabetic and antioxidant activities of ethanol extracts of *Aframomum danielli* seeds.

Materials and methods

Plant collection

Aframomum danielli was harvested and collected freshly from a farm in Owo, Ondo State and authenticated in the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo.

Chemicals and reagents preparation

All chemicals were of an analytical grade and are supplied from Sigma Chemical Co. USA. Distilled water was used in all biochemical assays.

Methods

Sample preparation of *Aframomum danielli*

The seeds of *Aframomum danielli* in a pod was purchased, dried, milled using a warring blender then, and sieved for analysis.

Extraction and preparation of *A. danielli* extract

A slight modification of Onoagbe and Esekheigbe (1999) method was used to prepare the ethanol extract of *Aframomum danielli*. Here, the powdered sample of the plant weighed 1000g was soaked in absolute ethanol for 72 hours in a glass container and covered with cheesecloth. The content was stirred several times a day for three days, then filtered through two layers of cheesecloth and later with a filter paper to completely remove residues. The filtrate was concentrated by a rotary evaporator and then evaporated to dryness by means of a freeze dryer. The dried extract was weighed and stored in an air-tight container and kept in the freezer for use.

Spectra studies

Gas chromatography-mass spectrometer (GC-MS) analysis

The Gas chromatography-mass spectrometer (GC-MS) analysis of the pulverized, extracts of *A. danielli* were performed using a GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a VF-5MS fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm), according to the method of Hossain *et al.* (2011) and Elizabeth (2013) . In GC-MS spectroscopic detection, an electron ionization system with ionization energy of Ev was used. Pure helium gas (99.999%) was used as a carrier gas at a constant flow rate of ± 1mL/min. Mass transfer line and injector temperature were set at 220°C and 290°C respectively. The oven temperature was programmed 50°C to 150°C at 3°C/min, then held isothermal for 10 minutes and finally raised to 300 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 µL was injected in the split mode with a split ratio 120:1.

Ultra violet (UV) ray Analysis

Ultraviolet (UV) spectra of the *A. danielli* extracts were measured on a Shimadzu UV-240 1PC spectrophotometer (Shimadzu Coorporation, Tokyo, Japan). Optical rotation was measured with a Horbia SEAP-300 automatic polarimeter (Horiba, Tokyo, Japan), and melting points were performed on melting point apparatus, XRC-1 (Sichuan University, Sichuan, China) according to the method of Bayoub *et al.* (2011).

Experimental Design

Phytochemical analysis quantitative method

The A. Danielli was subjected to quantitative analysis by standard methods. Sample was prepared from the various organic solvents for alkaloids Herbone (1973), Tannin (Van-Burden and Robison, 1981), Saponin (Obadoni and Ochuko 2001), Oxalate determination (Ejikeme *et al.,* 2014), Phytate (Yadav and Agarwala 2011).

Mineral analysis

Proximate analysis of components of *A. danielli* in a food is divided into six categories based on the chemical properties of the compounds and determined according to standard method of analysis adopted from (*AOAC.* 2016). The six are: moisture content, ash content, crude fat content, crude fibre content, crude protein content, carbohydrate content calcium, potassium, sodium, iron, and zinc.

In vitro antioxidant and anti-diabetic activities assays

The *in vitro* antioxidant and anti-diabetic activities of *A. danielli* was evaluated based on the estimation of the IC_{50} values for its inhibitory potentials with varying concentration of the extracts and fractions (0.125, 0.250, 0.500, 1.000, 2.000 mg/ml).

In vitro antioxidants studies assays and procedures

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the various extracts of alligator pepper *A. danielli* was determined by measuring their ability to decolorize the purple-coloured methanol solution of DPPH, as described by Baliyan *et al*. (2022).

Nitric oxide scavenging activity
The potential of alligator pepper *A. danielli* extracts to scavenge nitric oxide radical were determined according to the procedure reported by Garrat (1964).

Reducing power property
The reducing power of the extracts alligator pepper *A danielli* were assessed according to the method of Oyaizu (1986).

Metal chelating
Metal chelation ability of alligator pepper *A. danieli* extracts and fractions were assayed following the procedure of Dinis *et al.* (1994).

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging ability
The ability of the extracts and fractions of *A. danielli*) were carried out according to the procedure of Re *et al.* (1999).

Hydroxyl radicals (HR) evaluation
The ability of the various extracts of alligator pepper *A. danielli* to stop Fe^{2+}/ H_2O_2 induced decomposition of deoxyribose was assayed using the modified method of Oboh *et al.* (2007).

***In vitro* antidiabetic potentials**
The *in vitro* antidiabetic activity of alligator pepper *A. danielli* was evaluated by the inhibition of α-amylase and α-glucosidase activities based on the methods described below.

α-amylase inhibitory assay and kinetics
The α-amylase and α-glucosidase inhibitory activities of *A. danielli* were determined using the methods described by Elsnoussi *et al.* (2012) respectively.

α-glucosidase inhibitory assay and mode of inhibition
The α-glucosidase inhibitory activity of *A. danielli* was done according to the method of Elsnoussi *et al.* (2012).

Statistical analysis
Statistical analyses were done using GraphPad Prism 5 statistical package (GraphPad Software). One-way analysis of variance was used to analyze the data followed by Bonferroni test. Results were expressed as a mean ± standard error of the mean (SEM) of three replicates determination. Statistical significance of their mean values was considered at $p < 0.05$.

Results and Discussions

Phytochemicals, mineral analysis, proximate composition
The phytochemicals present in aqueous extract of *A. danielli* revealed the following in their respective proportions in g/100g sample, tannins (1.436 ± 0.007), flavonoids (0.102 ± 0.001), alkaloids (0.315 ± 0.049), phytate (1.207 ± 0.016), oxalate(3.377 ± 0.064), cyangenic glycoside (12.835 ± 0.163) was also recorded to be present in the extract (table 1). Also, the mineral analysis (table 2) carried out on our extract shows various minerals with significantly different values in this order, Ca (95.75±0.354), K (302.30±0.141), Fe (0.678±0.002), Na (19.750±0.071), Zn (2.063±0.018)

Table 1: Phytochemical screening of the aqueous extracts of *A. danielli*

Sample (g/100g)	**Value**
Tannins	1.436 ± 0.007
Flavonoids	0.102 ± 0.001
Alkaloids	0.315 ± 0.049
Phytate	1.207 ± 0.016
Oxalates	3.377 ± 0.064
Cyangenic Glycosides	12.835 ± 0.163

Result showing the presence of different phytochemicals in (g/100g) sample of *A. danielli* ethanol extract, + shows the presence of compound in the leaves extract.

Table 2: Mineral analysis of the *A. danielli* ethanol extracts

Sample	Value
Ca	95.75 ± 0.354
K	302.30 ± 0.141
Na	19.750 ± 0.071
Fe	0.678 ± 0.002
Zn	2.063 ± 0.018

Result showing the presence of the various minerals contained in *A. danieli* leaves with higher value obtained in this order, Ca, K, Fe, Na, Zn. ($p>0.05$)

The spectral analysis of *A. danielli* ethanol extract
The GC-MS analysis of *A. danielli* aqueous leaves extract revealed the presence of the following thirteen compounds and their retention time (table 3 and figure 1): 2-Butanone, 4-(4-

hydroxy-3-methoxyphenyl)- (10.823), 2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)-(10.892), Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester(13.358), benzenesulfonamide, N-(4-amino-2-methoxyphenyl)-(15.624), 1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one(16.122), 1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione(16.494), 4-Hydroxy-3-methoxybenzyl alcohol, di(2-methylpropyl) ether(18.045), ultra violet (UV) spectroscopy scan method (figure 2) study further revealed the various functional groups attached to some of the named compounds.

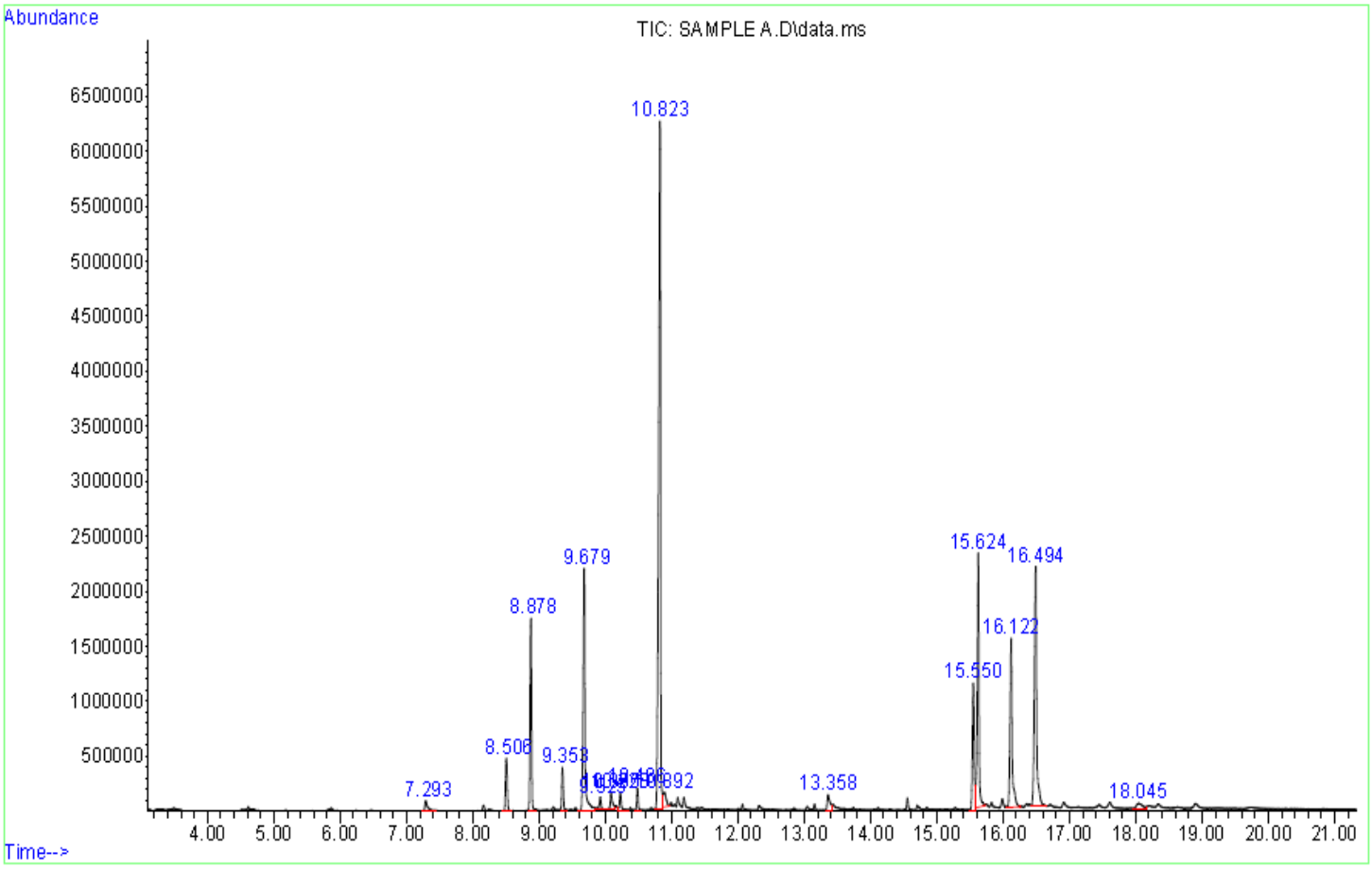

Figure 1: Gas chromatography mass spectrophotometer (GCMS) spectral analysis of aqueous extract of *A. danielli* using the scanning method

Table 3: Chemical compounds present in ethanol extract of *A. danielli* using GC-MS

Compounds	Retention time(RT)	Area
2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	10.823	31.54
2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)-	10.892	0.99
Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	13.358	1.16
benzenesulfonamide, N-(4-amino-2-methoxyphenyl)-	15.624	10.61
1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one	16.122	8.96
1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	16.494	13.81
4-Hydroxy-3-methoxybenzyl alcohol, di(2-methylpropyl) ether	18.045	0.77

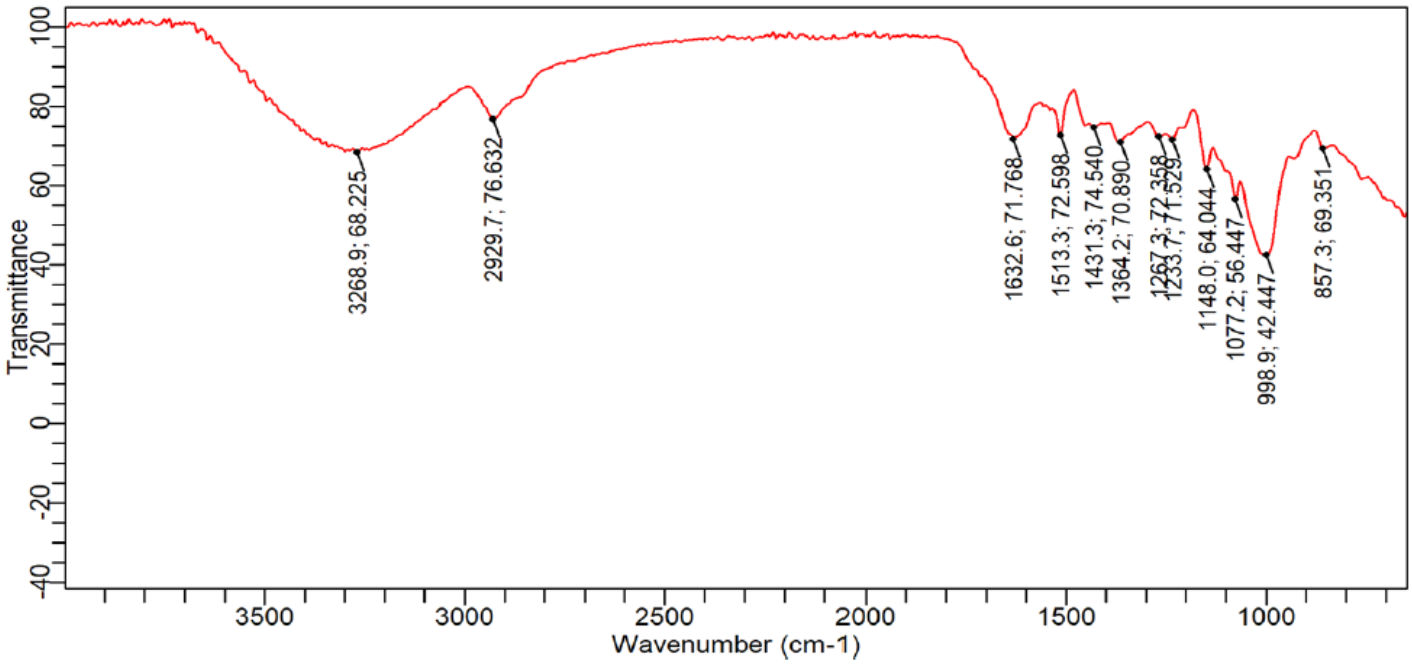

Figure 2: The ultra violet (UV) spectroscopy scan method of ethanol extract of *A. danielli* using Scan Spectrum curve

Values in the figure are the absorbance as represented by the scan spectrum curve for the aqueous extract of *A. danielli* using the ultra violet (UV) spectroscopy scan method.

***In vitro* Antioxidant and anti-diabetic studies**

In table 4, the DPPH radical scavenging ability of the *A. danielli* ethanol extract revealed that silymarin (control) had the highest scavenging ability at the highest concentration as shown by the IC_{50} value of (58.45±0.92), while the aqueous leaf extract showed lower scavenging ability with the IC_{50} (43.11±0.34) at the same concentration. The nitric oxide scavenging ability of the aqueous *A. danielli* leaves extract revealed that silymarin (control) (IC_{50} 4.46±0.77) had the highest scavenging ability at the highest concentration while IC_{50} aqueous leaves extract (51.92±0.36) had the lowest scavenging ability at the same concentration. The hydroxyl radical scavenging ability of the aqueous *A. danielli* leaves extract revealed that silymarin (control) IC_{50} (13.91±0.49) had the highest scavenging ability at the highest concentration while IC_{50} aqueous leaves extract (11.72±0.66) had the lowest scavenging ability at the same concentration.

The nitric oxide scavenging ability of the ethanol extract of *A. danielli* revealed that silymarin (control) (IC_{50} 4.46±0.77) had the highest scavenging ability at the highest concentration while IC_{50} aqueous leaves extract (51.92±0.36) had the lowest scavenging ability at the same concentration. The metal chelating ability of the *A. danielli* ethanol extract revealed that silymarin (control) (IC_{50} 21.00±0.28) had the highest scavenging ability at the highest concentration while IC_{50} ethanol extract *A. danielli* (20.27±0.17) had the lowest scavenging ability at the same concentration. The *A. danielli* ethanol extract inhibited α– amylase and α–glucosidase activities as shown in the Table 5 based on the IC_{50} values for α– amylase (3.81±0.32) and α–glucosidase (49.93±1.98) comparing these to our control (acarbose), our extracts showed significant difference ($p<0.05$) in inhibiting α–amylase and α– glucosidase (acarbose 3.10±0.85) and (46.06±1.35) respectively, this implies that our extract had the highest inhibitory effect.

Table 4: IC_{50} (mg/mL) values for the *in vitro* antioxidant assays of *A. danielli* ethanol extract

	IC_{50} (mg/ml)			
Assay	**DPPH**	**Nitric oxide**	**Hydroxyl**	**Metal chelating**
Silymarin	58.45 ± 0.92[a]	47.46 ± 0.77 [a]	13.91 ± 0.49 [a]	21.00 ± 0.28 [a]
***A. danielli* ethanol extract**	43.11 ± 0.34 [a]	51.92 ± 0.36 [a]	11.72 ± 0.66 [a]	20.27 ± 0.17 [a]

Values represent the *in vitro* antioxidant enzymes activities of extracts of *A. danielli* and are means ± SEM of 4 rats per group. Values carrying different letters are statistically significant different at $p < 0.05$. Where DPPH: α, α-diphenyl-β-picrylhydrazyl.

Table 5: IC_{50} (mg/mL) values for the inhibitory activities of *A. danielli* ethanol extract on the specific activities of α-amylase and α-glucosidase

Samples	IC_{50} (mg/ml)	
	α– amylase	α– glucosidase
Acarbose	3.10 ± 0.85 [a]	46.06 ± 1.35 [a]
***A. danielli* ethanol extract**	3.81 ± 0.32 [a]	49.93 ± 1.98 [b]

Values represent the *in vitro* antioxidant enzymes activities of extracts of *A. danielli* and are means ± SEM of 4 rats per group. Values carrying different letters are statistically significant different at $p < 0.05$

Discussion

The result of this work showed that the seed of *Aframommum danielli* ethanol extract is highly rich in phytochemicals flavonoids, phenolic compound tannins, saponin, terpernoids, cardiac glycosides and alkaloids which are solely responsible for its biological activities. This suggests that the plant extract is broad spectrum in activity (Oyagade, 1999). The efficacies of *A. danielli* defines its biological potentials due to the various compounds present in it as revealed through the gas chromatography mass spectrophotometer (GC-MS). The biological functions of flavonoid, Phenols and tannins includes protection against allergies, inflammation, free radicals platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu, 2004; 2005). Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic, antioxidants, anti-diabetic effects (Ajaiyieoba and Ekundayo, 1999), which complements the outcome of this study. They exhibit marked physiological activity when administered to animals. The high tannin content could be partly responsible for the hot, bitter and pungenty taste of *A. danielli* seed.

The presence of phenolic compounds in the seed of *A. danielli* indicates that this plant might be an anti-diabetic agent. Extracts from *A. danielli,* therefore, have potent anti-diabetic properties. Thus, it can ameliorate free radicals and its effects (Okwu, 2004). This finding supports the use of extract(s) of *A. danielli* in treating wounds especially wounds that are diabetic-related and not only heals fast but also prevents the formation of infections (Alaje *et al.,* 2014). The presence of phenol further indicated that the seed of this plant could act as anti inflammatory, anti clotting, antioxidant, immune enhancers and hormone modulators. This plant also has quantity of saponin content. Some of the general characteristics of saponin include formation of foam in aqueous solutions, hemolytic activity cholesterol binding properties and bitterness (Igwe *et al.*, 1999). This may be the reasons behind the use of the extracts of this plant in reducing elevated blood sugar and other related diseased conditions (Okwu, 2004).

Conclusion

The plant *A. danielli* can be of immense use in phytomedicine and in health care delivery system starting from the developing countries. Further studies on more effective method of extracting the necessary constituents, processing and purification measures would be necessary. It can be concluded from this study that the extracts from the seed of *A.danielli* showed antioxidant and anti-diabetic activities.

References

Ajaiyieoba, O.E & Ekundayo, O. (1999). Essential oil constituents of *Aframomum dannieli* (Roscoe) K. Schum. seeds (alligator pepper) from Nigeria. *Flavour and Fragrance J.* 14(2): 109-111.

Alaje, D.O., Owolabi, K.T., Olakunle, T.P., Oluoti, O.J. & Adetuberu, I.A. (2014). Nutritional, Minerals and Phytochemicals composition of Garcinia cola (Bitter cola) and Aframomum dannieli (Alligator pepper). IOSR *Journal of Environmental Science, Toxicology and Food Technology*, 8(1):86.

Antonioni, A., Fantini, C., Dimauro, I., & Caporossi, D. (2019). Redox homeostasis in sport: do athletes really need antioxidant support? *Res. Sports Med.* 27, 147–165.

AOAC International. Official Methods of Analysis of AOAC International. 20th ed. Gaithersburg, MD, USA: AOAC International; 2016. p. 3172.

Baliyan, S. Mukherjee, R. Priyadarshini, A. Vibhuti, A. Gupta, A. Pandey, R.P. & Chang C. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical

Analysis of *Ficus religiosa. Molecules*. 27(4): 1326.

Devasagayam, A., Manisha, M., Priyanjali, D.,Jayant, L., Saroj, G., & Thomas, P. (2007). *J, clin. Biochem Nutr*. 40:163.

Dinis, T.C.P., Madeira, V.M.C. & Almeida, M.L.M. (1994). Action of Phenolic derivates (acetoaminophen, salycitate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315: 161-169.

Ejikeme, C.M. Ezeonu, C.S. & Eboatu, A.N. (2014). "Determination of physical and phytochemical constitutents of some tropical timbers indigenous to Niger delta Area of Nigeria".*European Scientific Journal.* 10(18): 247–270.

Elizabeth, T., Aneesh, T.P., Della, G.T. & Anandam, R. (2013) GC-MS analysis of phytochemical compounds present in the rhizomes of Nervilia aragoana GAUD. *Asian J Pharm Clin Res*. 6: 68-74.

Elsnoussi, A.H.M., Mohammad, J.A.S., Lee, F.A., Amirin S, Sue, H.C., & Soo, C.T. (2012). Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. *BMC Complement Altern Med.* 12:176.

Garratt, D.C. (1964). *The quantitative analysis of Drugs*. (Vol. 3.). Japan: Chapman and Hall ltd.

Guariguata, L., Whiting, D.R., Hambleton, I.,Beagley, J., Linnenkamp, U., & Shaw, J.E. (2014). Diabetes. *Res. Clin. Pract.* 103: 137.

Harbone, J.B. (1973). Phytochemical Methods: A guide to modern techniques of plants analysis, London, UK: Chapman and Hall.

Hossanin, M.A.,Nahar, N.,Kamal, & Islam, M.N. (1995).Nutrient digestibility coefficients of some plants and animal proteins for tilapia (Oreochromis mossambicus).*Journal Aquaculture in the Tropics,* 7;257-266.

Igwe, S. A., Emeruwa, I. C. & Modie, J. A. (1999). Ocular toxicity of Aframomum dannieli (alligator pepper) on healthy igbos of Nigeria. *Journal of Ethnopharmacology,* 65(3):203-205.

Jabeen, S., Salim, M. & Akhtar, P. (2004). Study on feed conversion ratio of major carp (*Cirrhinus mrigala*) fingerlings fed cotton seed meal, fishe meal barly. *Pakistan Veterinary Journal.* 24(1) 42-26.

Lawal, O.; Kasali, A.A.; Opoku, A.R.; Ojekale, A.B.; Oladimeji, O.S.; & Bakare, S. (2015). Chemical composition and antibacterial activity of essential oil from the leaves of Aframomum melegueta (Roscoe) K. Schum from Nigeria. J. Essent. Oil Bear. *Plants,* 18: 222–229.

Mclaughlin, J.L., Chang. C.J,. & Smith, D.L. (1991). "Bench-top" bioassays for the discovery of bioactive natural products; an update. in: atta-ur-rahman, editor. amsterdam: Studies in natural products chemistry, *Elsevier* Science Publishers.

Ngane, R. (2013). The chemical composition and antibacterial activities of the essential oils from three Aframomum species from Cameroon, and their potential as sources of (E)-(R)-nerolidol. *Nat. Prod. Comm.* 8: 829–834.

Obadoni, B.O. & Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and delta States of Nigeria. *Global Journal Pure Appl. Sci.*, 8:203-208.

Oboh, G., Nwanna, E.E. & Elusiyan, C.A. (2006). Antioxidant and AntimicrobialProperties of *Telfairia occidentalis* (FlutedPumpkin) Leaf Extracts. *J. of Pharmacologyand Toxicology,* 1(2): 167-175.

Okwu, D.E. (2004). Phytochemicals and vitamin content of Indigenous spices of South Eastern Nigeria. *Journal of Sustain Agricultural environment,* 6: 30-34.

Okwu, D.E.(2005). Phytochemical and Vitamin content of indigenous spices of Southern Nigeria . *Journal of Sustainable Agriculture and. Environment,* 6(1) 30-37.

Onoagbe, I.O. & Esekheigbe, A. (1990): Studies on the anti-diabetic properties of Uvaria chamae in Streptozotocin-induced diabeteic rabbits. *Biokemistri,* 9(2), 79-84.

Oyagade, J.O., Awotoye, O.O., Adewunmi, J.T., & Thorpe, H.T., (1999) Antimicrobial activity of some Nigerian medicinal Plants, screening for antibacterial activity. *Journal of Bioscience research Communication,* 11:193-197.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition*, 44: 307.

Re, R., Pellegrini, N., Proteggente, A., Pannala A, Yang M. & Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine,* 26: 1231-1237.

Van-Burden, T.P. & Robinson, T. (1981). *The biochemistry of alkaloids* (2nd Ed.). Springer, Heidelberg, New York.

Yadav, R.N.S. & Agarwala, M. (2011). Phytochemical analysis of some medicinal plant, *Journal of Phytology*, 3(12): 10-14.