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Inhibitory Properties of Polyphenolic Phytochemicals of *Cola nitida* on Carbohydrate Hydrolyzing Enzymes of Wistar Rat In-Vivo

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Abstract

Polyphenolic phytochemicals of *Cola nitida*, with their potential therapeutic ability can modulate metabolic and transcriptional expression of proteins and enzymatic activities that culminate to hyperglyceamic effects. This study investigated the hyperglyceamic effects of polyphenolic phytochemical extracts of *Cola nitida* in Wistar rat *in-vivo* and established a parallel inhibition of the enzymes (α-amylase and α-glucosidase) in their activities as the concentration increased. On administration of the extracts, the activity of α-amylase was inhibited from control, 32 ± 0.00 to 16 ± 0.02u/L as the concentration increased. Also, the activity of α- glucosidase was inhibited from 17 ± 0.01 to 10 ± 0.00u/L in relation to the concentration of the extracts. The inhibitory effect of *Cola nitida* on carbohydrate hydrolyzing enzymes delayed glucose utilization resulting to elevated glucose concentration from 6.07 ± 0.02 to 9.11 ± 0.01 mMol/L. The composition of the polyphenolic phytochemicals in the *Cola nitida* analyzed with gas chromatographic techniques was found to contain; apigenin, 6.0 ± 0.01; catechin, 7.1± 0.01; epicatechin, 4.8 ± 0.02; narigenin, 4.9 ± 0.10 mg/100mg and these are the active ingredients of the bioactive compounds that are responsible for the hyperglyceamic effects of *Cola nitida*.

Keywords: *Cola nitida*, polyphenolics, glucose, α-amylase, α- glucosidase.

1.0: INTRODUCTION

The use of plants and its products in scientific study of traditional medicines is called ethno-pharmacology. *Cola nitida* is a plant native in West Africa employed in ethno-medicine against various ailments including diabetes. It is an important nut in Africa which belongs to the family Malvaceae, subfamily Sterculioideae with many species (Adebola and Morakiny, 2005, Opeke, 2005, Onomo *et al*., 2006) which are of commercial importance. *Cola nitida* is easily distinguishable by its nuts of two cotyledons, while *Cola acuminata* has three to six cotyledons. *Cola nitida* and *Cola acuminata* when chewed has a bettering-sweet taste that lasts long and keeps one salivating. The nuts are of commercial importance as they are also used in wine, chocolate and many beverages as flavouring agents. The kola industry provides employment and income opportunities to people involved in the harvesting, processing, packaging and transportation of nuts (Asogwa *et al*., 2012, Dah-Nouvlessounon *et al*., 2015). The crop has socio-cultural importance in Nigeria especially during traditional rites (Akpertey *et al*., 2017) such as burial and wedding ceremonies.

In Nigeria, *Cola nitida* is eaten to stimulate and keep awake without being fatigue. Besides the fact that kola nuts contain caffeine and act as a stimulant and anti-depressant, they are also thought to reduce hunger and aid digestion (Sofowora, 1993). Kola nuts are offered to visitors and prayer is made before it is broken. In fact, it is a revered nut in Nigeria. Besides the ceremonial uses, many Africans consume kola nuts regularly, even daily, for the medicinal effects such as diabetes (Sofowora, 1993). More recently, kola nut extracts have become popular in Europe and North America as a natural or alternative medicine for management of diabetes (Sofowora, 1993).

Kola nuts are nutritious and contain high levels of phytochemicals which are the bioactive compounds that constitute the hallmark of its ethno-medicine. It is rich in polyphenolics and other essential bioactive compounds (Meda *et al*., 2005, Kelley *et al*., 1994, Zar, 1999). Polyphenolic compounds are widely distributed in edible plants and have been suggested to protect against a variety of diseases (Cheplick *et al*., 2010). Recent investigations suggest that polyphenolic components of higher plants may act as antioxidants or via other mechanisms that prevent disease processes

(Oboh and Rocha, 2007). Recent findings have also demonstrated that polyphenols cross intestinal barriers and are sufficiently absorbed to have the potential to exert biological effects (Sofowora, 1993). They are also important for improving the nutritional qualities, by imparting colors, flavors and tastes (Oboh and Rocha, 2007).

Bioactive compounds (phytonutrients) such as carotenoids and phenolic acids are health-promoting compounds that act against cardiovascular diseases and various types of cancer (Oboh *et al*., 2012; Olas, 2018). Phenolic compounds exert a potent antioxidant activity and are analgesic, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-microbial, anti-obesity, cardioprotective, hypotensive and neuroprotective (Plazas *et al*., 2013). The presence of kolanin and theobromine makes the nuts of kola suitable for development of new pharmaceuticals and foods (Fereday *et al*., 1997). Also, the volatile oil from *C. nitida* exhibits antioxidant properties and involves in apoptosis and therefore has potential to be an important medicinal resource (Fontenot *et al*., 2007; Solipuram *et al*., 2009).

Insulin is known for its hypoglyceamic effect. Impairment in glucose utilization from food molecules (carbohydrate, protein and fatty acid) as a result of insufficiency in insulin results to a metabolic disease known as diabetes mellitus. Diabetes mellitus (DM) is associated with too much sugar in the blood (hyperglyceamia) and not enough is stored for future use. The consequence in this impairment leads to demand for energy in form of ATP from non-carbohydrate sources such as proteins and lipids. In untreated DM, gluconeogenesis reduces citric acid intermediates. Less pyruvate is made available for acetyl-CoA synthesis and more of later is derived from lipids. The acetyl-CoA from excessive lipid mobilization which causes lipemia does not enter the citric acid cycle because cycle intermediates have been drawn for gluconeogenesis. The acetyl-CoA thus accumulates to form acetoacetatyl-CoA and further accumulation to β -hydoxyl- β -methylglutaryl-CoA (HMG-CoA). These are strong acids. The ribosomes of a diabetic patient exhibits reduced ability to incorporate amino acids into ribosomal proteins. Protein synthesis is therefore depressed.

Type 2 diabetes is characterized by insulin and pancreatic β-cell dysfunctions which manifested in hyperglyceamia. *Cola nitida*, with its polyphenolic phytochemicals reduce or inhibit glucogenic enzymes, the α-glucosidase and α-amylase leading to a reduction in blood glucose turnover. Inhibition of these enzymes delays carbohydrate metabolism leading to a reduction in the rate of glucose absorption (Kwon *et al*., 2007; Ranilla *et al*., 2010). Thus, this study is sought to analyze the hyperglyceamic effect of polyphenolic phytochemicals in *Cola nitida*.

1.1: Aim and objectives

This study was aimed at substantiating the inhibitory properties of Cola nitida on carbohydrate hydrolyzing enzymes which culminate in hyperglyceamia of Wistar rat in-vivo, with the following objectives:

1) To extract the polyphenolic phytochemical content of C. nitida.
2) To estimate the blood glucose of the Wistar rats.
3) To assay the hydrolyzing enzymes; α-Amylase, α-Glucosidase.

2.0: MATERIALS

The kola nuts for this research were purchased in market at Owerri, Imo State, Nigeria and the botanical name, Cola nitida, was given to it at the Botany Department of the Federal University of Technology, Owerri, Imo State, Nigeria. The kola nuts were pelleted, chopped into small chips and dried at room temperature on the laboratory bench. The dried chips were blended into powdered form.

2.1: Animal treatment

Seven adult Wistar rats of different sexes weighing about 180 – 210kg were purchased from the animal house of the Department of Zoology, University of Nigeria, Nsukka and acclimatized for one week. During acclimatization period, the animals were fed with rat pellets, water, libitum and maintained under standard husbandry conditions in accordance with the recommended international standard (National Research Council, 1988).

2.2: METHODS

2.2.1: Polyphenolic Phytochemical Extraction

The method of Oboh et al., (2007) was used for the extraction of the polyphenolic phytochemicals. 100 ml of 80% acetone was used for the phenolic extraction from fifteen (15g) grams of the powdered seed. Appropriate dilutions of the extracts with 2.5 mL 10% Folin-Ciocalteau's reagent (v/v) and neutralized with 2.0 mL of 7.5% sodium carbonate (Singleton *et al*., 1999) was carried out. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in the spectrophotometer. Thereafter, the animals were orally administered with uniform concentration of the extract (100mg/kg) for 4 weeks. They were sacrificed and blood sample collected for analysis of the aforementioned objectives.

2.2.2: Glucose Estimation

The method of Ochei and Kolhtkar (2000) was used in the estimation of glucose using Randox diagnostic kits.

2.2.3: Enzyme Assay

2.2.3.1: α-Amylase (EC 3.2.1.1)

Cola nitida extracts, 100 µl and 300 µl of 0.02 mol/l sodium phosphate buffer (pH 6.7 with 0.006 mol/l NaCl) containing porcine pancreatic α-amylase (0.5

mg/ml) were incubated at room temperature, 25 °C, for 10 min. Then, 300 µl of 1% starch solution in 0.02 mol/l sodium phosphate buffer (pH 6.7 with 0.006 mol/l NaCl) was added to each tube. The reaction mixtures was incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was boiled, cooled and incubated at room temperature and the absorbance taken at 540nm wavelength. The α-amylase inhibitory activity was expressed as percentage inhibition (Worthington, 1993):
% Inhibition = $[(Abs_{Control} - Abs_{Samples})/Abs_{Control}] \times 100$

2.2.3.2: α-Glucosidase (EC 3.2.1.20)

Dilutions of the extracts, 200 µl, and 50 µL of α-glucosidase solution (1.0 U/ml) in 0.1 mol/l phosphate buffer (pH 6.7) were incubated at 25 °C for 10 min. Then, 50 µl of 3.0 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/l phosphate buffer (pH 6.7) was added, incubated at room temperature for 5min and the absorbance was taken at 405nm wavelength.

The α-glucosidase inhibitory activity was expressed as percentage inhibition (Apostolodis *et al*., 2007):
% Inhibition = $[(Abs_{Control} - Abs_{Samples})/Abs_{Control}] \times 100$.

2.3: Statistical Analysis

The results were expressed as mean ± standard deviation (SD). All results were compared to control. Comparisons between the concentrations and control were made by using Statistical Package for Social Sciences (SPSS) version 20 and One-way Analysis of Variance (ANOVA). Differences at ($p < 0.05$) were considered significant.

3.0: RESULT

3.1: Table 1.0: Polyphenolic constituents of *C. nitida* (mg/100g)

Apigenin	6.0 ± 0.01
Catechin	7.1 ± 0.01
Epicatechin	4.8 ± 0.02
Narigenin	4.9 ± 0.10

The concentrations of polyphenolic phytochemicals are presented in Table 1.0.

3.2: Glucose estimation

Following the analysis, blood glucose concentration increased from control; 6.07 ± 0.02 to 9.11 ± 0.01 mMol/L.

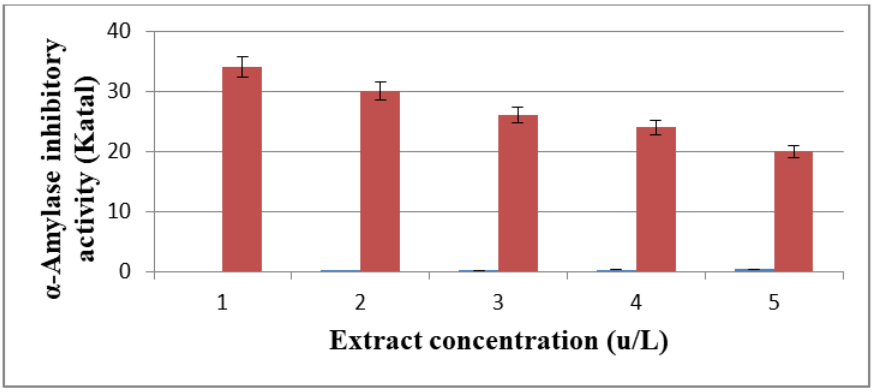

Fig 1: α-Amylase inhibitory activity

3.2: The concentration of the extract, *C. nitida*, paralled the inhibition of **α-Amylase.**

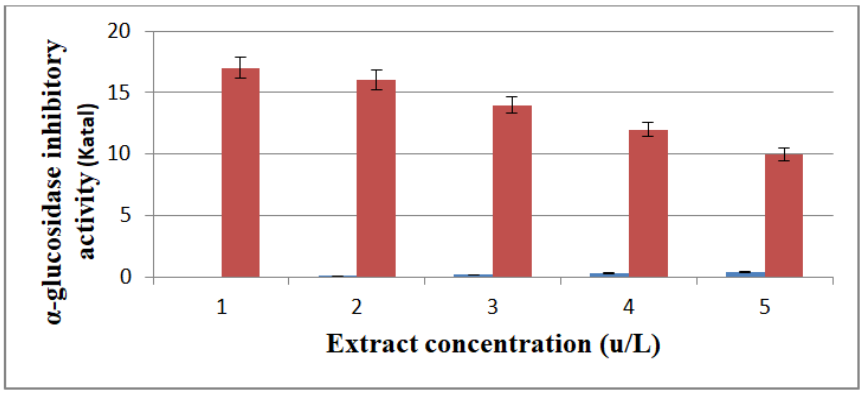

Fig 2: α-Glucosidase inhibitory activity

3.3: The inhibitory effect of the extract, *C. nitida*, on α-Glucosidase is in consonance with the concentration as presented in Fig 2.

4.0: DISCUSSION

Man has over the years been in the discovery of medicinal plants, ethno-medicine. Polyphenolic phytochemicals being the bedrock of ethno-pharmacology and ethno-medical practice is one of the constituents of *Cola nitida*.

Regulation of blood glucose is imperative and in this study, the hyperglyceamic effect of *Cola nitida* was investigated in Wistar rat in-vivo. The phytochemical constituents and the hyperglyceamic factors were also investigated. The analysis established the constituents of polyphenolic phytochemicals are; epigenin (6.0 ± 0.01), catechin (7.1 ± 0.01), epicatechin (4.8 ± 0.02) and narigenin (4.9 ± 0.10) mg/100g and these are essentially responsible for the hyperglyceamic factor of *Cola nitida*. Its effect in the delay of blood glucose metabolism was accentuated as it was increased from 6.07 ± 0.02 to 9.11 ± 0.01mMol/L. Blood glucose regulation is imperative in the management of diabetes mellitus as sustained glycemic control decreases the risk of developing cardiovascular complications (Nathan *et al*., 2005; Bash *et al*., 2008). The marked delay in blood glucose metabolism in this study following administration of *Cola nitida* corroborated with the previous studies, thus demonstrating its hyperglycemic effects (Adaramoye, 2006). The mechanisms of hyperglycemic effect of *Cola nitida* might not be unconnected with the combination of its stimulating action on the pancreatic β cells coupled to release of insulin and also an insulin independent effect and extra-pancreatic action which involves glucose utilization in extra-hepatic tissues (Mezei *et al*., 2003; Pinent *et al*., 2004).

Enzyme inhibition has been a therapeutic measure and inhibitors of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia in diabetes mellitus patients. In this study, *Cola nitida*, due to its polyphenolic flavonoid content (apigenin, naringenin, epicatechin and catechins) inhibit carbohydrate hydrolyzing enzymes, α-amylase from 32 ± 0.00 to 16 ± 0.02 Katal and α-glucosidase, from 17 ± 0.01 to 10 ± 0.00 Katal. The inhibition of these enzymes delays carbohydrate digestion, prolongs the overall carbohydrate digestion time and a reduction in the rate of glucose absorption (Kwon *et al*., 2010). This finding is in tandem with previous research on inhibition of α-amylase by flavonoids (Ranilla *et al*., 2010; El-Kaissi and Sherbeeni, 2011; Cheplick *et al*., 2010).

Similar inhibitory effect was also observed in α-glucosidase by the polyphenolic extract of *Cola nitida* and this could be attributable to the presence of flavonoids (Pata and Chua, 2011). This is in consonance with the reports of inhibitory effects of phenolic compounds from medicinal plants, herbs and spices in Latin America against key enzymes relevant to hypoglycemia (Ranilla *et al*., 2010; Ye *et al*., 2010).

Furthermore, the hyperglycemic effect of flavonoids can be mediated through an increase in hepatic glucose storage by stimulating the action of glycolytic and glycogenic enzymes or by inhibiting glucose-6-phosphatase. This consequently results in the uptake of glucose into cells and the reduction in the blood glucose level through the up-regulation of glycogen formation, down-regulation of the rate of glycogen breakdown, and glucose synthesis (Naik *et al*., 1999; Waltner *et al*., 2002; Srkhail *et al*., 2007).

5.0: CONCLUSION

The research has proved that *C. nitida*, due to its polyphenolic phytochemicals has inhibitory effect on carbohydrate hydrolyzing enzymes. This effect is accentuated in low glucose metabolism, elevated glucose concentration and reduced rate of glycolysis, hence its hyperglyceamic effects.

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REFERENCES

- Adaramoye, O., & Adeyemi, E. (2006). Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from Garcinia Kola in streptozotocin–induced diabetes mellitus rats. *Journal of Pharm Pharmacology*, *58*(1), 121-128.
- Adebola, P., & Morakinyo, J. (2005). Chromosome numbers of four nigerian species of *Cola Nitida*. Schott. & Endlicher (Sterculiaceae). *Silvae Genet*ics, *54*(1–6), 42–44.
- Akpertey, A., Dadzie, A. M., Adu-Gyamfi, P. K. K., Ofori, A., & Padi, F. K. (2017). Effectiveness of juvenile traits as selection criteria for yield efficiency in kola. *Sci Hortic-Amsterdam*, 216, 264–271.
- Apostolidis, E., Kwon, Y. I., & Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Inn Food Science Emerging Technology*, 8, 46–54.
- Asogwa, E., Otunde, A., Oluyole, K., Ndubuaku, T., & Uwagboe, E. (2012). Kolanuts production, processing and marketing in the South Eastern states of Nigeria. *Journal of Agriculture and Environmental Sciences*, *12*(4), 463–468.

- Bash, L. D., Selvin, E., Steffes, M., Coresh, J., & Astor, B. C. (2008). Poor glycemic control in diabetes and the risk of incident chronic kidney disease even in the absence of albuminuria and retinopathy: Atherosclerosis Risk in Communities (ARIC) Study. *Archives of Internal Medicine*, *168*(22), 2440-10.
- Cheplick, S., Kwon, Y. I., Bhowmik, P., & Shetty, K. (2010). Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. *Bioresource Technology*, 101, 404–413.
- Dah-Nouvlessounon, D, Adjanohoun, A., Sina, H., Noumavo, P. A., Diarrasouba, N., & Parkouda, C. (2015). Nutritional and anti-nutrient composition of three kola nuts (*Cola nitida*, *Cola acuminata* and *Garcinia kola*) produced in Benin. *Food and Nutrition Science*, *6*(15), 1395–1407.
- El-Kaissi, S., & Sherbeeni, S. (2011). Pharmacological management of type 2 fiabetes mellitus: an update. *Current Diabetes Review*, *7*(6), 392-405.
- Fereday, N., Gordon, A., & Oji, G. (1997). Domestic market potential for tree products from farms and rural communities: Experience from Cameroon. Greenwich: Natural Resources Institute (NRI), The University of Greenwich.
- Fontenot, K., Naragoni, S., Claville, M., & Gray, W. (2007). Characterization of bizzy nut extracts in estrogen-responsive MCF-7 breast cancer cells. *Toxicology and Applied. Pharmacology*, *220*(1), 25–32.
- Kelley, W. I., Coffey, D. L., & Mueller, T. C. (1994). Liquid chromatographic determination of phenolic acids in soil. *J Ass Anal Commun Int*, 77, 805–809.
- Kwon, Y. I., Apostolidis, E., Kim, Y. C., & Shetty, K. (2007). Health benefits of traditional corn, beans and pumpkin: *in vitro* studies for hyperglycemia and hypertension management. *J Med Food*, 10, 266–275.
- Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem*istry, 91, 571–577.
- Mezei, O., Banz, W. J., Steger, R. W., Peluso, M. R., Winters, T. A., & Shay, N. (2003). Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese zucker rats and murine RAW 264.7 cells. *Journal of Nutrition*, *133*(5), 1238-1243.
- Naik. S. R., Fliho, J. M. B., Dhuley, J. N., & Deshmukh, A. (1999). Probable mechanism of hypoglycaemic activity of bassic acid, a natural product isolated from *Bumelia sartorum*. *Journal of Ethnopharmacology*, 33, 37-44.
- Nathan, D. M., Cleary, P. A., Backlund, J. Y., Genuth, S. M., Lachin, J. M., Orchard, T. J., Raskin, P., & Zinman, B. (2005). Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *New English Journal of Medicine*, 353, 2643-2654.
- National Research Council. (1988). Use of laboratory animals in biomedical and behavioral research. Washington, DC: The National Academy Press.
- Oboh, G., Akinyemi, A., & Ademiluyi, A. (2012). Inhibition of α-amylase and α-glucosidase activities by ethanolic extract of *Telfairia occidentalis* (fluted pumpkin) leaf. *Asian Pac Journal of Tropical Biomedicine*, *2*(9), 733–738.
- Oboh, G., Puntel, R. L., & Rocha, J. B. (2007). Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinese*, Habanero) prevents Fe^{2+}-induced lipid peroxidation in brain - *in vitro*. *Food Chemistry*, 102, 178–185.
- Oboh, G., & Rocha, J. B. (2007). Polyphenols in red pepper [*Capsicum annuum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. *European Food Research and Technology*, 225, 239–247.
- Ochei, J., & Kolhatkar, A. (2000). Lipids and Lipoprotein. In: Medical Laboratory Science: Theory and Practice, McGraw Hill Publishing Co. Ltd., New Delhi, 311-347.
- Olas, B. (2018). Berry phenolic antioxidants–implications for human health? *Front Pharmacology*, 9, 78-83.
- Onomo, P. E., Niémenak, N., & Ndoumou, D. O. (2006) Isoenzyme variability of three cola (*Cola acuminam* (Pal. de Beauv, Schott and Endlicher), *Cola nitida* ((Vent) Schott and Endlicher) and *Cola anomala* (Schott and Endlicher)) germplasm in Cameroon. *Pak Journal of Biological Sciences*, *9*(3), 391–398.
- Opeke, L. (2005). Tropical commodity tree crops. Spectrum Books Limited.
- Patra, J. C., & Chua, B. H. (2011). Artificial neural network-based drug design for diabetes mellitus using flavonoids. *Journal of Computational Chemistry*, *32*(4), 555–567.
- Pinent, M., Blay, M., Bladé, M. C., Salvadó, M. J., & Arola, L. (2004). Grape seed-derived procyanidins have an antihyperglycemic effect in streptozotocin-induced diabetic rats and insulinomimetic activity in insulin-sensitive cell lines. *Endocrinology*, 145, 4985-4990.
- Plazas, M., López-Gresa, M. P., Vilanova, S., Torres, C., Hurtado, M., & Gramazio, P. (2013). Diversity and relationships in key traits for functional and apparent quality in a collection of eggplant: Fruit phenolics content, antioxidant activity, polyphenol oxidase activity, and browning.

Journal of Agriculture and Food Chemistry, *61*(37), 8871–8879.

- Ranilla, L. G., Kwon, Y. I., Apostolidis, E., & Shetty, K. (2010). Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource and Technology*, *101*(12), 4676–4689.
- Sarkhail, P., Rahmaipour, S., Fadyevatan, S., Mohammadirad, A., Dehghan, G., & Amin, G. (2007). Antidiabetic effect of *Phlomis anisodonta*: effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. *Pharmacol Research*, 56, 261-266.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Cioalteau reagent. *Methods in Enzymology*, 299, 152–178.
- Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Nigeria, Ibadan: Spectrum Books Ltd; p. 289.
- Solipuram, R., Koppula, S., Hurst, A., Harris, K., Naragoni, S. and Fontenot, K. (2009). Molecular and biochemical effects of a kola nut extract on androgen receptor-mediated pathways. *Journal of Toxicology*, 10.1155/2009/530279.
- Waltner-Law, M. E., Wang. X. L., Law, B. K., Hall, R. K., Nawano, M., & Granner, D. K. (2002). Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *Journal of Biological Chemistry*, 277, 34933-34940.
- Worthington, V. Worthington (1993). Enzyme Manual. Freehold: Worthington Biochemical Corp. Alpha amylase; pp. 36–41.
- Ye, X., Song, C., Yuan, P., & Mao, R. (2010). α-Glucosidase and α-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. *Chinese Journal of Natural Medicine*, 8, 349–352.
- Zar, J. H. (1999). Biostatistical analysis. USA: Prentice Hall PTR; p. 663.