Original Research Article

Combining aqueous extracts of *Vernonia amygdalina* and *Tamarindus indica* inhibits two digestive enzyms and improves hematological profile of streptozotocin-induced diabetic rats.

ABSTRACT

Aims: Many strategies have been put in place to stop diabetes and its related complications: the use of plants has proved to be less costly and with few side-effects. Among these plants *Vernonia amygdalina* and *Tamarindus indica* are used because of their richness in bioactive compounds, notably phenols and dietary fiber. The aim of this work was first to evaluate the effect of combining aqueous extracts of *Vernonia amygdalina* leaves and *Tamarindus indica pulp* on two energy metabolism enzymes (alpha-amylase and pancreatic lipase), and secondly to assess the effect of combining these extracts on the haematological profile of streptozotocin-induced diabetic *wistar* rats.

Methodology: *Vernonia amygdalina* leaves and *Tamarindus indica* pulp have subjectof (Replace with 'was subjected to') aqueous extraction, the two extracts obtained were mixed *in vitro* in the presence of the two enzymes and their inhibitory power evaluated; streptozotocin was used to induce diabetes in *Wistar* rats which were then treated with our extracts and the combination of these; their blood was collected and hematological analysis carried out (Through which method. Please state the method's reference).

Results: Our findings revealed that at 100 mg/l, the mixture showed a 23.56±4.6% inhibition of alpha-amylase. The percentage inhibition of pancreatic lipase varied depending on the extract concentration from 8.81±1.90 at 62.5µg/ml to 31.95±1.90 at 1000µg/ml, and the mixture also improved hematological parameters in diabetic rats.

Conclusions: combining these two extracts makes it possible to increase the inhibitory power with respect to these enzymes and improves the hematological profile, this could therefore be beneficial for the non drug management of diabetes.

Key words: type 2 diabetes mellitus, *Vernonia amydalina*, *Tamrindus indica*, α-amylase, pancreatic lipase

1. INTRODUCTION

Everywhere in the world today, we can observe an increase in the prevalence of non-communicable diseases including high blood pressure, obesity and diabetes. The World Health Organization defines diabetes as a disorder that occurs when a person's body is unable to produce a hormone called insulin, or when the body does not properly use the insulin it produces **(WHO, 2016)**. This disorder results in abnormally high blood sugar levels, and the clinical diagnosis is based on fasting blood sugar levels above 1.26 g/l on two occasions. Diabetes also leads to anemia and immunosuppression, manifested by disturbances in the hematological profile of patients **(Pretorius *et al.*, 2015)**. Lack of insulin production leads to type-1 diabetes, while bad utilization of the insulin produced by the body is responsible for type-2 diabetes, the most common type with over 80% of diagnosed cases. Type 2 diabetes appears as a complication of obesity, to which it is closely linked. From 108 million in 1980, the number of people suffering from diabetes has risen to 422 million in 2014, and the International Diabetes Federation predicts over 629 million diabetics worldwide by 2045 if the current rate of growth continues, representing an overall increase of 54% **(IDF, 2019).**These alarming figures make diabetes a real problem.The first weapons used to combat diabetes were essentially synthetic drugs. Although the modes of action are different, these drugs have (please add 'helped') in common to induce a reduction in glycemia and improve patient's physiological parameters (This is a categorical statement thus requires referencing). These drugs include insulin secretors, biguanides, α-glucosidase inhibitors, thiazolidine-diones and insulin therapy; however, diabetic patients face other problems (like.....so so and so), prompting researchers to look to other sources of molecules with anti-diabetic activity that would be less costly, easily accessible and have few or no side effects. Plants and functional foods would be a good alternative, acting in several different ways. For example, work by **Nivesh *et al.* (2020)** revealed that the aqueous extract of *Tamarindus indica* pulp exerts its anti-diabetic potential by acting as an inhibitor of pancreatic α-amylase and lipase; in 2020, **Waldemar *et al*.** showed that ethanolic extracts of *Vernonia amygdalina* were able to protect the pancreas. **Hoang *et al*. (2019)** reported that saponins extracted from the leaves of *Vernonia amygdalina* were able to act as α-aamylase and alpha glucosidase inhibitors. **Ouédraogo *et al.* (2020)** showed that *Tamarindus indica* was able to act as an alpha amylase inhibitor. However, there are no works in the literature mentioning the combination of *Vernonia amygdalina* and *Tamarindus indica* for anti-diabetic activity or an inhibitory effect on these important enzymes of carbohydrate metabolism. In the same (view change to 'vein), this work was therefore undertaken with the aim of evaluating the inhibitory potential of the combination of aqueous extracts of *Vernonia amygdalina* and *Tamarindus indica* for anti-diabetic activity.

2. MATERIAL AND METHODS

2.1. Chemicals

Alpha amylase, pancreatic lipase and, streptozotocin were purchased from Sigma-Aldrich, Germany. The extracts and reagents were prepared on the day of the experiments. Streptozotocin as a disease inducer was given to the rats intraperitoneally (ip) while the extracts and the extract mixture were administrated by oral gavage.

2.2. Plant material

Vernonia amygdalina leaves were collected in the West region of Cameroon and *Tamarindus indica* fruit samples were collected in the North Cameroon region. These samples were identified in the national herbarium and sent to the Research Unit of Biochemistry, Medicinal Plants, Food Sciences and Nutrition (RUBPMAN laboratory of the Biochemistry)

2.3. Extraction

Aqueous extract of *Vernonia amygdalina* leaves and *Tamarindusi ndica* pulps were obtained according to the method described by **Razali *et al.* (2012)**, by maceration of powders (10g) of samples into 100 ml of water for 24 hours with gentle stirring, after which the mixtures were filtered using a Whatman N°1 filter paper. The resulting filtrates were dried at 45 ˚C in an air oven to obtain the aqueous extract. The extracts were weighed to calculate the yield, then a mixture of extracts was made using 1/3 extract of *Vernonia amygdalina* leaves and 2/3 *Tamarindus indica*; mixture and extracts were stored in the freezer at -4°C for later use.

Yield (%) = Mass of extract×100 / Mass of powder

2.4. Laboratory animals

Four-week-old *Wistar* rats were obtained from the Department Animal Centre and allowed to be accustomed to the new environment for 1 week. They were maintained in accordance with the guidelines of the OECD and were randomly distributed into (height please correct to eight) groups of seven animals each (including two controls). The animals were individually housed under controlled temperature (25°C) and lighting (12:12-hours light-dark cycle) and had free access to water and diet. The test groups and the positive control were fed a high-fat high sucrose diet (17.6% fat and 7% sucrose-enriched) while the negative control received a basal diet. The high-fat-high-sucrose diet-induced obesity was carried out for twelve (12) weeks and diagnosed by a body mass index greater than 0.68 **(Noveli *et al*., 2007).** These obese animals were administered with a single dose of 45 mg/kg of streptozotocin. Three days after the injection of streptozotocin, animals having a fasting blood glucose concentration higher than 200 g/l were considered diabetic **(Chang, 2000)** and used for the treatment (please add 'procedure that lasted for twenty-one day (21 days).

2.5. Alpha amylase Inhibition

The *in vitro* α-amylase inhibitory activity of extracts and mixture of extracts was carried out using the method described by **Bernfeld (1995).** In brief, 0.02–4 µL of the different extracts reacted with 200 µL of α-amylase enzyme, and 100 µL of 2mm of phosphate buffer (pH, 6.9). After 20-min pre-incubation, 100 µL of 1% starch solution was added. The same was performed for the controls. After incubation of 5min, 500 µL of dinitrosalicylic acid reagent was added to both the control and test. They were kept in boiling water bath for 5min, the absorbance was recorded at 540nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the following formula: % Inhibition = [(Control - Test)/Control]×100

2.6. Pancreatique (change to Pancreatic) lipase Inhibition

The modified method of **Beisson *et al.* (2000)** was used to assess lipase inhibitory activity. This technique was adopted to measure the ability of the extracts tested to reduce the production of free fatty acids in a reaction medium containing an emulsified substrate and pancreatic lipase. Released fatty acids were titrated by adding 0.1 N sodium hydroxide to the reaction medium. A decrease in the volume of sodium hydroxide compared with the control corresponds to the percentage of enzyme inhibition by the extract.

To (please add 'the') test tubes containing 100 µl of extract solutions at concentrations of 1000, 500 and 250 µg/ml, prepared in distilled water, we added 1 ml of 10% olive oil emulsion (10 ml olive oil in 90 ml of 10% gum Arabic **(Rathelot *et al.,* 1975)** (100 g g gum arabic in one liter of boiled water, then filtered) and 500 µl of pancreatic lipase solution (2 IU/ml) prepared in phosphate buffer (0.2 M, pH 8) containing 1 mm $CaCl_2$. The whole was incubated for 30 minutes at 40°C in a water bath, then 20 ml of distilled water and 3 drops of phenolphthalein were added. The mixture was titrated with 0.1 N sodium hydroxide solution. Pink coloration indicated turning, which persisted for 5 min. The percentage of lipase inhibition by the extracts was determined by the following formula:

Where:

- Vc = volume of NaOH in the control tube without extract,

-Ve = volume of NaOH in the test tube containing extract. (please complete this calculation formula)

2.7. Treatment of rats with extracts and mixture of extract

The rats were randomly divided into eight groups of 7 rats each as follows: Group I and Group II received saline and served as the normal and positive control. Group III received glibenclamide (80 mg/kg) and served as a model group. Group IV received aqueous extract of *Vernonia amygdalina* (500 mg/kg), groups V received aqueous extract of *T.indica* (500 mg/kg), group VI received (association Please change this to 'combination) of *V. amygdalina* and *T. Indica* (500 mg/kg please change this to 250mg/kg each), group VII received association of *V. amygdalina* and *T. indica*(250 mg/kg Please check and recast this combination) and group VIII received both association of *V. amygdalina* and Tamarindusindica(500 mg/kg in what combination volume) and glybenclamide (80 mg/kg) why this additional 80mg/kg).

2.8. Animal sacrifice, blood collection and hematological tests

Twenty-four hours after the last administration, the animals were sacrificed under light anesthesia using ketamine. Dissecting instruments were washed with 5% nitric acid and rinsed several times with distilled water before use. Blood samples collected in EDTA tubes were used to perform a haemogram on an impedance haematology machine (QBC Autoread Plus, UK). The counting principle is based on impedance variation and flow cytometry to determine the size and type of blood cells. Flow cytometry measures on a suspension of particles (cells, bacteria, parasites, beads) the individual characteristics of each particle such as size, shape and complexity of any component or function that can be detected by a fluorescent compound. Cells in suspension pass one by one past one or more laser beams, and detectors pick up signals emitted by each cell. Each time a cell passes through the aperture, the electrical resistance increases. This increase is translated into electrical pulses, the height of which is directly proportional to the cell volume. Cells emit signals, either naturally or after treatment, which are analyzed by the computer linked to the cytometer, enabling the leukocyte formula to be established, giving the percentages of the different types of leukocytes.

The number of red blood cells was determined by the total number of pulses recorded. The hematocrit level was then deduced from the formula:

Hematocrit = red blood cell × mean corpuscular volume /10.

Hemoglobin was determined spectrophotometrically (525 to 550 nm) after lysis of the red blood cells.

2.9. Statistical analysis

Data were expressed as the mean ± standard error of the mean. After the analysis of variance, the comparison of means between different groups was carried out by the Waller-Duncan test using SPSS version 26.0 software. P values =0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Extraction yields

After obtaining (our change to 'the') various extracts, the extraction yields evaluated were **9.98%** for *Vernonia amygdalina* and **17.26%** for *Tamarindus indica*.

3.2. α-amylase inhibitory activitivity

The following figure 1 shows the ability of extracts and mixture of extracts to inhibit α-amylase at 100mg/l. It appears that the two extracts (are please change to 'were') able to act as α-amylase inhibitors but the percentage of inhibition is lower than that of the reference drug used (Acarbose). However, we can observe that the (association please change to 'combination') of the two extracts gives a percentage of inhibition higher than any of the two extracts.

The combination of aqueous extracts of *Vernonia amygdalina* and *Tamarindusindica* inhibits α-amylase and this can be attributed to the bioactive compounds present in our two (samples, Please change to 'materials') notably phenols and dietary fibres. Indeed, work by **Quan *et al.* (2019)** reveals that the position of the hydroxyl and methoxy groups of the phenolic compounds contained in these extracts could be at the origin of the antioxidant and alpha amylase inhibitory activities.

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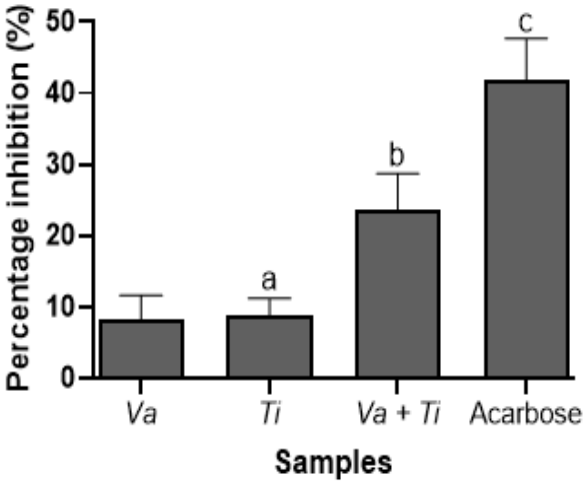

Figure 1: Effect of extracts and mixture of extracts in vitro on α-amylase activity in comparison with acarbose.Va: *Vernoniaamygdalina*, Ti: *Tamarindusindica*, Va+Ti: Mixture. Samples with different letters are significantly different at p=0.05.

3.3. Pancreatic lipase inhibitory activity

The ability of our different samples to act as an inhibitor of pancreatic lipase is reported on the following figure 2. It appears that the percentage of inhibition increase with the concentration of extracts and mixture; it also appears that the combination of the two extracts can increase the inhibitory ability of the extracts. The main function of pancreatic lipase is to catalyse hydrolysis reactions **(Pandey *et al*., 1999)** ; the pancreatic lipase inhibitory activity observed here can be attributed to several classes of compounds, including catechins, coumarins, quercetins and tannins, which act as pancreatic lipase inhibitors **(Dunaif *et al.,*1981),** it can also be attributed to dietary fiber which have the capacity to limit lipolysis thus increasing the fecal excretion of lipids **(Kundu *et al.,* 2014),** extracts of *Vernonia amygdalina* and *Tamarindus indica* are known to be rich dietary fiber and many phenolic compounds **(Sadiq *et al.*, 2016).** Phenols and fibers therefore act various ways, including limiting glycation of red blood cells and reducing apoptosis of leukocytes **(Kundu *et al.,* 2014).** These results corroborate those obtained by **Auger *et al.* (2019),** who demonstrated that phenols from extracts of nine Niger plants were capable of inhibiting pancreatic lipase.

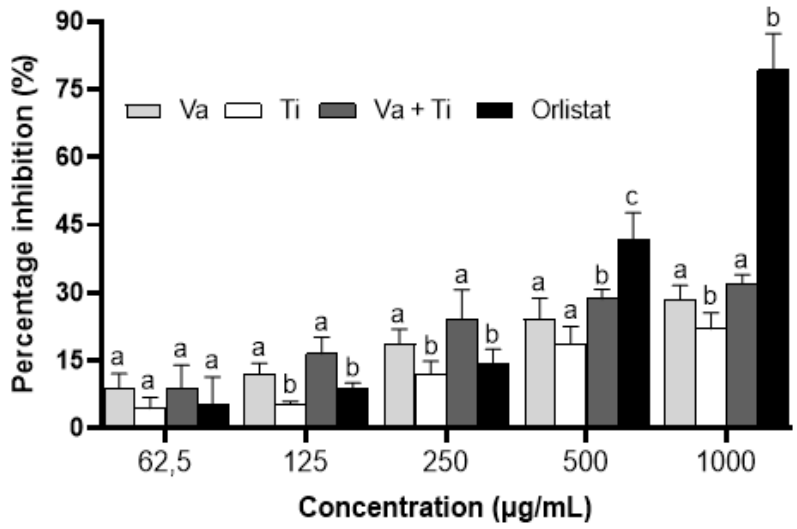

Figure 2:c
Effect of extracts and mixture of extracts *in vitro* pancreatic lipase activity, in comparison with Orlistat. Va: *Vernonia amygdalina*, Ti: *Tamarindus indica*, Va+Ti: Mixture. Samples with different letters are significantly different at p=0.05.

3.4. Effect of plant extracts and the mixture of extracts on hematologic parameters

Table 1 shows the number of white blood cells, the percentage of lymphocytes and, the number of granulocytes in the blood of animals treated with extracts and a mixture of extracts. It appears that the combination of extracts can increase the number of white blood cells and granulocytes.The number of red blood cells, hemoglobin and, the hematocrin have also been evaluated; the results are grouped in the following table 2. (We please change to 'it can be') observed that the number of red blood cells and hemoglobin of animals treated with extracts and the mixture of extracts (kindly add 'were') nearly the same as those of animals treated with the reference drug (Glibenclamide). The mixture of extracts improved hematological parameters in treated animals, reflecting the ability of the compounds in our mixture to reduce immunological complications associated with diabetes.These results corroborate those of **Woumbo *et al*. (2022),** who reported that the phenols in a nutraceutical formulated from extracts of three plants improve the hematological profile of streptozotocin-induced diabetic rats.

Table 1: White blood cells, lymphocyte and granulocytes in the blood of animals after treatment.

	WBC*10^9(/L)	LYM (%)	GRAN*10^9(/L)
Ti	3.1 ± 0.30^b	74.5 ± 1.10^b	2.16 ± 0.20^b
Va	2.43 ± 0.15^a	86.4 ± 3.01^d	1.6 ± 0.26^a
Ti+Va 500	4.56 ± 0.35^c	81.5 ± 1.24^d	2.3 ± 0.26^b

Ti+Va 250	2.16± 0.49[a]	78.63±1.40[c]	1.63±0.25[a]
Ti+Va+Gly	3.5±0.10[b]	83.83±4.7[d]	2.2±0.36[b]
Glib	5.2±0.26[d]	77.63 ± 2.47[c]	2.13± 0.32[b]
C(+)	2.53±0.25[a]	69.34±2.72[a]	1.33 ± 0.15[a]
C(-)	7.06 ± 0.47[e]	82.7 ±4.94[d]	3.96 ±0.70[c]

WBC: White blood cells, LYM: lymphocytes, GRAN: granulocytes, Va: *Vernonia amygdalina*, Ti: *Tamarindus indica*, Va+Ti: Mixture. Glib: Glibenclamide, C(+): positive control, C(-): negative control, Values in the same colum, with different letters are significantly different at p=0.05.

Table 2 : Red blood cells, hemoglobin and hematocrit in the blood of animals after treatment.

	RBC*10^{12}(/L)	HGB (g/dl)	HCT (%)
Ti	8.1± 0.17	14.73±0.37	46.6±0.10
Va	8.27±0.21	15.26 ±0.30	50.2± 0.72
Ti+Va 500	7.25±0.12	13.5± 0.95	43.33±2.49
Ti+Va 250	8.08 ±0.50	14.2± 0.81	45.2 ± 3.87
Ti+Va+Gli	8.083± 0.11	14.7±0.36	47.36± 0.80
Gli	7.8±0.23	14.4 ± 0.36	46.7± 2.74
C(+)	27.36 ±0.30	13.36±0.49	42.8± 0.28
C(-)	8.46 ±0.24	15.36 ±0.55	47.46±1.11

White bloodcells, HGB : Hemoglobin, HCT : Hematocrin ; Va: *Vernonia amygdalina*, Ti: *Tamarindus indica*, Va+Ti: Mixture, Glib: Glibenclamide, C(+) : positive control C(-) : negative control.

4. CONCLUSION

The aim of the present work was to demonstrate that combination of aqueous extract of *Vernonia amygdalina* and *Tamarindus indica* was able act as good inhibitors of two keys enzymes of energetic metabolism (namely alpha amylase and panreatic lipase) and also promote good effect on the hematologic profile of streptozotocin-induced diabetic rats. It was found that the mixture of these extracts contains many bioactive compounds with better activities than extracts taken separately. Further detailed studies with purified compounds may provide evidence to effectively control diabetes

REFERENCES

- World Health Organization. (2016).Aide-mémoire N°312, http://www.who.int/gho/fr/consulted in avril 2022.
- Pretorius, E., Bester, J.,Vermeulen, N., Alummoottil,S., Soma, P.,Buys, A.V. and Kell, D.B. (2015). Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. *CardiovascDiabetol*;14(8):30.
- FID: International Diabete Federation: IDF diabetes Atlas.6th edition .2019.
- Nivesh, K.R.,Anitha, R. and Ezhilarasan, D. (2020). Aqueous extract of *Tamarindus indica* fruit pulp exhibits antihyperglycaemic activity. *Avicenna J Phytomed*.,10(5): 440-447 Please write in full as 'Avicennna Journal of Phytomedicine
- Waldemar, S.,Chrismis, N.G., Linda, C., Nyoman, E. L., Sukirman, L.(2020).Pancreas Protective Effect of Ethanolic Extract Vernonia amygdalina Delile on Rats Induced Doxorubicin.3rd International Conference on Mechanical, Electronics, Computer, and Industrial Technology (MECnIT). Kindily italicise as it is a conference paper and quote the conference venur, country and pages of this paper on the proceedings.
- Hoang, L.T., Vinh, L.B., Thi,L.L.,Cuong, P.V., Masayoshi, A., Ha, T.P.,et al .(2019). In vitro pls remove study on α-amylase inhibitory and α-glucosidase of a new stigmastanetype steroid saponin from the leaves of Vernonia amygdalina. https HYPERLINK "https://doi.org/10.1080/14786419.2019.1607853"://doi.org/10.1080/14786419.2019.1607853
- Ouédraogo, R.J., Somda, M.B.,Ouattara, L.,Windmi K., Paulin, O. and Ouédraogo, G.A. (2020). Evaluation of the antioxidant and α-amylase inhibitory activities of mitragynainermis (willd) O. kuntze and tamarindus indica linn. *Journal of Experimental Biology and Agricultural Sciences*.Volume 8(5): 676 – 682. doi:10.18006/2020.8(5).676.682. Re-cite this doi please
- Razali, N., Mat-Junit, S., Abdul-Muthalib, A.F., Subramaniam, S. and Abdul-Aziz, A.(2012). Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of *Tamarindus indica.* L. *Food Chem.*131(2):441–448 DOI10.1016/j.foodchem..09.001 Cite in full please
- OECD: Guidelines for chemicals trials: repeated dose oral toxicity studies for 28 days in rodents http://www.oecd-ilibrary.org
- Novelli, E.,Diniz, Y., and Galhardi, C.(2007). Anthropometrical parameters and markers of Obesity in rats. *Lab Animals.* 41: 111–119,

- Chang, K.J. (2000). Effect of taurine and beta alanine on morphological changes of pancreas in streptozotocin induced diabetic rats. *Adv in exp med and biol*483: 571–577, Cite in full
- Bernfeld, P., Colowick, I.N. and Kaplan, NO. (1955). Amylase, a and b in Methods in Enzymology. Academic Press, New York, NY, USA (pp. 149–158).
- Beisson F., Tiss A., Rivière C. and Verger R. (2000). Methods for lipase detection and assay: A critical review. *Eur. J. Lipid Sci. Technol,*.2: 133-153. Kindly write in full
- Rathelot, J., Julien, R., Canioni, P., Coeroli,C. and Sarda, L. (1975). Studies on the effect of bile salt and colipase on enzymatic lipolysis. Improved method for the determination of pancreaticlipase and colipase. *Biochimie,* 57: 1117-1122.
- Quan, N.V.,Xuan, T.D., Tran, H.D., Thuy, N.T., Trang, L.T., Huong, C.T. and Tuyen P.T.(2019). Antioxidant, α-amylase and α-glucosidase inhibitory activities and potential constituents of *Canarium tramdenum* bark. *Journal of Molecular Catalysis*.Vol. 78, 24(3), 605.
- Pandey, A., Benjamin,S., Soccol, C.R., Nigam, P., Krieger, N. and Soccol, V. (1999).The mecanism of microbial lipases in biotechnology. *Biotechn and ApplBiochem*. 29 (2): 119-131. Cite in full
- Dunaif, G. and Schneeman, B.(1981). The effect of dietary fiber on human pancreatic enzyme activity in vitro. *Am. J. clin. Nutr*.,.34: 1034-1035.
- Kundu, D., Mandal, T., Nandi, M., Osta, M., Bandyopadhyay, U. and Ray, D. (2014)Oxidative stress in diabetic patients with retinopathy. *Ann Afr Med*. Jan-Mar; 13(1):41-6
- Sadiq, I.S., Duruminiya, N.I., Balogun, J.B., kwada, D. and Izuagie, T. (2016). Nutritional and Anti-nutritional Value of Tamarind Fruit (*Tamarindus indica*). International *J of Appl Res and Techn*. 5(3): 50 – 56
- Auger, M., Chantale, F., Pierre, H.S. and Alain, C.(2019).An ethnobotanical study of antidiabetic medicinal plants used in Niamey (Niger) with an *in vitro* pancreatic lipase inhibition assay, mémoire de master. Université de Montréal. 2019.
- Woumbo, C.Y., Kuate, D., Metue, D.G. and Womeni H.M. (2022). Antioxidant and antidiabetic activities of a polyphenol rich extract obtained from *Abelmoschus esculentus* (okra) seeds using optimized conditions in microwave assisted extraction (MAE). *Front. Nutr.* 9:1030385. doi: 10.3389/fnut.2022.1030385 Please cite appropriately