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Antioxidant Activities and FT-IR of *Azanza garckeana* Leaves Extracts

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Abstract

The objective of this study is to evaluate the antioxidant activities of three extracts of *Azanza garckeana* leaves. *Azanza garckeana* fruits, seeds and roots have been reported to cure cough, liver disorders, abscess, diabetes, infertility and mental sickness. Natural antioxidants found in plants help to reduce oxidative stress in the body that lead to variety of health issues, including heart disease, diabetes, muscular degeneration, and cancer. The antioxidant activities of *Azanza garckeana* leaves extracts were determined by 2,2-diphenyl-1-picryhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) Methods. *Azanza garckeana* leaves were extracted with three solvents of increasing polarity of n-hexane, ethyl acetate and methanol and their antioxidant properties were tested. DPPH results showed that methanol and ethyl acetate extracts have excellent antioxidant activities when compared with the standard oxalic acid. Methanol has the highest antioxidant activity, followed by ethyl acetate and then n-hexane extract with IC_{50} values of 1.17µg/ml, 2.23µg/ml and 17.83µg/ml, respectively. Ferric reducing antioxidant power also indicated that ethyl acetate and methanol extracts have higher reducing power than n-hexane extract when compared with ascorbic acid (AA). This may be as a result of higher concentrations of flavonoid and

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phenol in the extracts. The leaves extracts of *Azanza garckeana* contains potent antioxidant agents supporting its folkloric uses in the treatment of diseases.

Keywords: Antioxidant, *Azanza garckeana,* 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP).

INTRODUCTION

Scientific studies for years have shown that different parts of plants such as the leaves, fruits, roots stem barks and seeds are source of many phytochemicals which demonstrate antioxidant activities (1). Antioxidant found naturally in plants help to prevent or minimize oxidative stress in the body. Because of the continual usage of oxygen, the body is constantly creating free radicals. These free radicals cause cell damage in the body and also lead to variety of health issues, including heart disease, diabetes, muscular degeneration, hypertension, stroke and cancer (2, 3). The antioxidative effect of particular part of the plant is mainly due to more phenolic contents (4). In traditional medicine, *Azanza garckeana* is used to cure some diseases and ailments (5). It was also confirmed as the herbal treatment for Coughs, chest pains, infertility, menstrual irregularities, painful menstruation, sexually transmitted infections, miscarriage and hepatic impairments done by the plant (6).Extracts from the plant have been evaluated for antimicrobial, anti-inflammatory, antimalarial, anti-infertility, antiarthritic, and anti-diabetic.

Plate 1: *Azanza garckeana* Plant

MATERIALS AND METHODS

Reagents and solvents

The solvents used are n-hexane, ethyl acetate, and methanol from Loba chemie limited, india. 2, 2-diphenyl-1-Picryhydrazyl (DPPH), disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, potassium chloride potassium ferricyanide, trichloroacetic acid, and Ferric Chloride.

Instruments

Soxhlet apparatus, Micro-plate reader (UT-200A full wavelength micro-plate reader) spectrophotometer, Cary 630 Agilent spectrophotometer glass wares (beakers, conical flasks, measuring cylinder, glass rod), micro pipettes.

Plant Collection and Identification

The plant sample (Leaves of *Azanza garckeana*) were collected in the month of January, 2022, at Tula village, Kaltungo local Government Area of Gombe State. The plant was identified at the Department of Plant Science/Botany, Faculty of Science, Gombe State University (specimen number GSUH198).

Plant Preparations and Extraction

The Leaves obtained were air dried in the laboratory at room temperature and then pulverized using mortar and pestle and then sieved. The extraction was carried out using soxhlet extraction with solvents of increasing polarity; n-hexane, ethyl acetate and methanol for six (6) hours each. The filtrates were allowed to evaporate. The extracts obtained were weighed and stored for further analysis.

Antioxidant Assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity of *Azanza. garckeana* Extracts

DPPH free radical scavenging activity: The antioxidant activity of *Azanza garckeana* was determined using 2,2-diphenyl-1-picryhydrazyl (DPPH) inhibition method as described by (7, 8, 9).

The test solutions were prepared by dissolving 30.0mg of each extracts (n-hexane, ethyl acetate and methanol) in 3ml of methanol to give corresponding stock concentrations of 10mg/ml. final concentrations of 1000, 500 ,250, 125, 62.5, 31.5, 15.6 and 7.23µg/ml were prepared by serial dilutions. 2,2-diphenyl-1-picryhydrazyl (DPPH) molar concentration of 0.076 mM was prepared. The three extracts were pipetted (40µL each) into micro-plates and 160µL of prepared DPPH was added to all the samples in the micro-plates, incubated for 30 minutes in the dark at room temperature. Then the absorbance at λ_{max}517nm was measured with a micro-plates reader spectrophotometer. IC_{50} value was calculated. The IC_{50} value states the concentration of the sample solution needed for free radical

scavenging by 50%. Absorbance values of the samples were expressed as radical scavenging activity (%RSA) using the formula described by (7,8,9).

- %RSA = (A_1-A_2/A_1) x100
- Where, A_1 is the absorbance of DPPH solution (control)
- A_2 = Absorbance of samples/standard
- The IC_{50} values were determined by probit analysis using SPSS 23.

Ferric Reducing Antioxidant Power (FRAP) of *Azanza. garckeana* Extracts

Ferric reducing antioxidant power of *Azanza garckeana* was evaluated by dissolving potassium ferricyanide $(K_3Fe(CN)_6)$ 1.0g in deionized water (100ml), trichloroacetic acid (10g) was dissolved in 100ml of deionized water, 0.1g of ferric chloride was dissolved in 100ml of deionized water, 0.1g of ascorbic acid was dissolved in 100ml of deionized water and sodium phosphate buffer of pH 7.4 was prepared (10). Samples (30mg) was dissolved in methanol (3ml) to give concentration of 10mg/ml. Different concentrations of 2000,1000,500,250 and 125 mg/ml of the samples were pipette into various vials, 1.25ml of sodium phosphate buffer of pH 7.4 and 1.25ml of potassium ferricynanide were pipette into the vials. The reaction mixture was vortexed well and then incubated at 30^0C for 20 minutes using vortex shaker, at the end of the incubation 1.25ml of 10% trichloro acetic acid was added to the mixture and 1.25ml of deionized water and 0.5ml of 0.1% ferric chloride were added (10).

The solution change to blue and the colour solution was read at 700nm against the blank with reference to standard using UV spectrophotometer. Ascorbic acid was used as positive control.

FT-IR Spectroscopic Measurement of *Azanza garckeana* Extracts

The FT-IR measurement of *Azanza garckeana* extracts were carried out using Cary 630 Agilent spectrophotometer.

RESULTS

DPPH Radical Scavenging Potential of *Azanza garckeana* Leaves Extract

Tables 1 and 2 showed antioxidant potentials of the leaves extracts of *Azanza garckeana* based on DPPH radical scavenging expressed as percentage inhibition as IC_{50} values in

µg/ml respectively.The antioxidant activities of the tested samples follows the order Methanol extract has the highest activity, followed by Ethyl acetate extract and n-hexane has the least.

Table 1: Percentage Radical Scavenging Activity of DPPH Assay

	Percentage radical scavenging activity (%)			
CONC (µg/ml)	**AGHX**	**AGEA**	**AGME**	**OXALIC ACID**
1000.00	93.01	96.86	94.85	96.15
500.00	90.18	91.50	93.93	95.97
250.00	76.46	89.93	93.09	95.25
125.00	61.18	89.68	91.14	94.83
62.50	55.46	84.18	80.67	94.86
31.25	54.22	74.20	72.14	93.47
15.63	51.41	64.69	78.94	93.03
7.81	48.93	69.20	69.81	92.76

Table 2: DPPH IC_{50} Values of the Extracts

EXTRACTS	**IC_{50} (µg/ml)**
AGHX	17.83
AGEA	2.23
AGME	1.17
Oxalic Acid	<0.01

Ferric Reducing Potential of *A. garckeana* Leaves Extract

Ferric reducing antioxidant power (Figure 1) indicates that ethyl acetate extract (AGEA) and methanol extract (AGME) have higher reducing power than n-hexane extract (AGHX).While the standard ascorbic acid (AA) demonstrated highest reducing potentials among the tested samples.

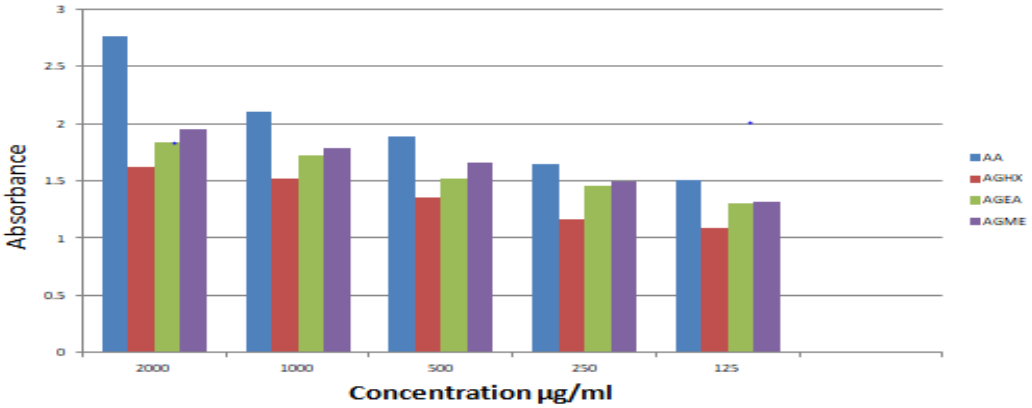

Figure 1: Ferric Reducing Antioxidant Power (FRAP) of the Extracts

FT-IR Spectroscopic Analysis of *Azanza garckeana* Leaves Extracts

The FT-IR spectra of n-hexane, ethyl acetate and methanol leaves extracts of *Azanza garckeana* are shown in figures 2 to 4

The IR spectrum of n-hexane extract (figure 2) showed strong peak at 2854 and 2921cm^{-1} for C-H stretch of CH_3, CH_2, and CH, the peak in the region of 1737cm^{-1} is assignable to carbonyl carbon stretch (C=O), peaks at 1249, 1171 and 1089cm^{-1} indicate C-O stretch.

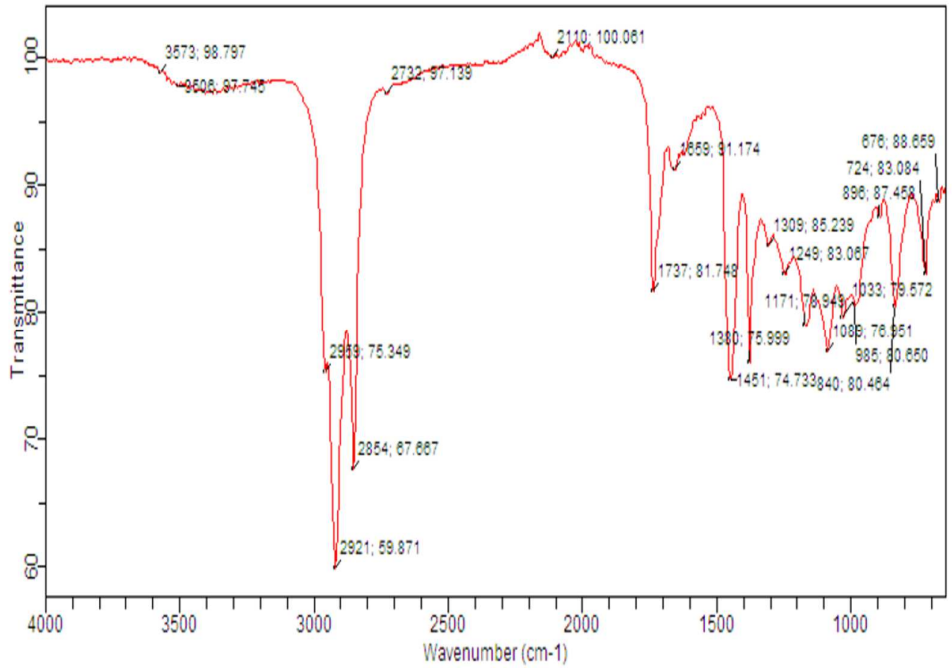

Figure 2: FT-IR Spectrum of n-hexane Extract (AGHX)

The FT-IR spectral analysis of ethyl acetate extract (figure 3) showed a broad band peak at 3327cm^{-1} that corresponds to hydroxyl group (OH) stretch, the peaks at 2921 and 2851cm^{-1} correspond to C-H stretch, absorption peak at 1618 and 1737cm^{-1} indicate carbonyl carbon bond, 1033 and 1246cm^{-1} are for C-O stretching.

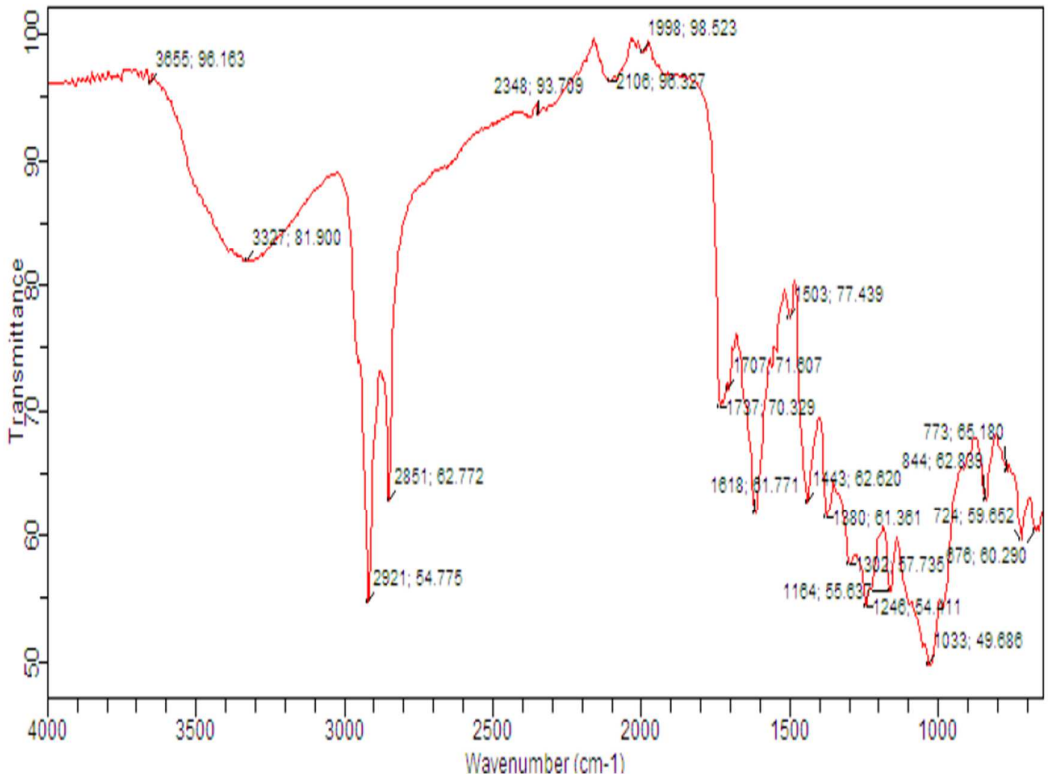

Figure 3: FT-IR spectrum of ethyl acetate extract (AGEA)

The FT-IR spectral analysis of methanol (figure 4) confirmed the presence of different functional groups at respective wave number. A very broad band peak in the region of 3234cm^{-1} is assignable to hydroxyl group (OH) of carboxylic acid, 2921 and 2854cm^{-1} are for C-H stretch vibration of alkyl methyl and methylene group, 1611 and 1707cm^{-1} are for carbonyl carbon (C=O) stretching and 1037 and 1171cm^{-1} indicates C-O stretch.

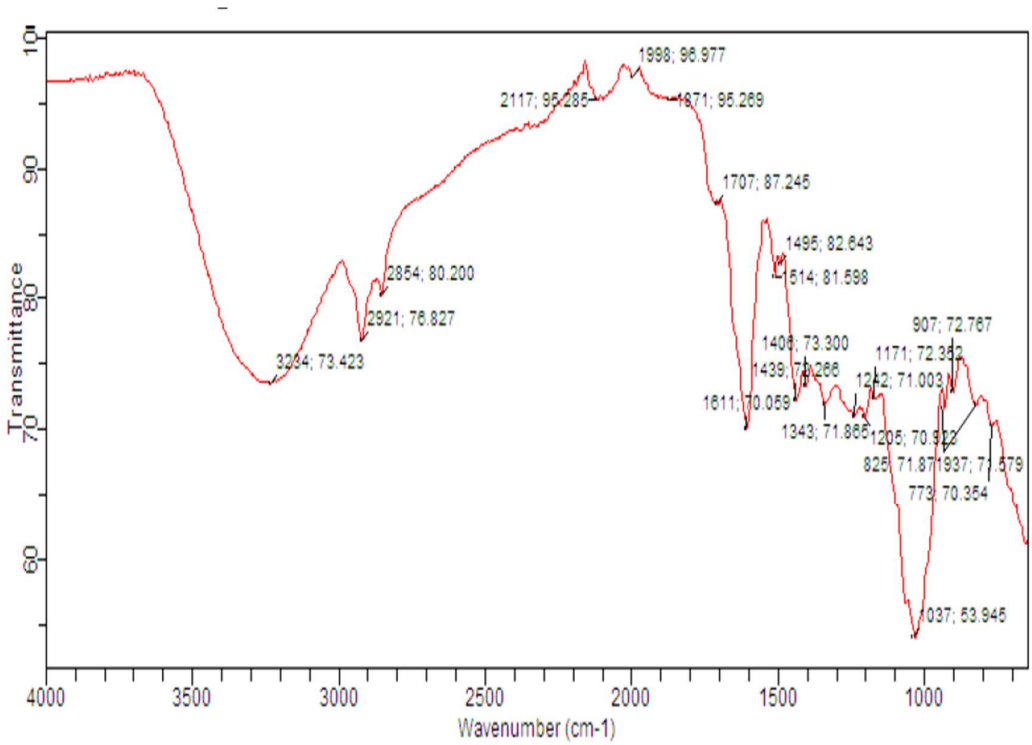

Figure 4: FT-IR spectrum of methanol extract (AGME)

DISCUSSION

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay is considered as a valid, accurate, easy and economical method to evaluate radical scavenging activity of antioxidants, since radical compound is stable and need not to be generated (11). Being a stable free radical, DPPH takes on an electron or hydrogen atom to transform into a stable diamagnetic molecule. When antioxidants are present, the rich purple hue (color) of a prepared DPPH solution diminishes as DPPH free radicals are quenched and transformed into the yellow product 2,2-diphenyl-1-picrylhydrazine (1). In this study, the antioxidant scavenging activities of n-hexane, ethyl acetate and methanol extracts were determined using two methods: 2,2-diphynel-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP). The DPPH radical scavenging activity of the analyzed extracts was expressed based on percentage inhibition and IC_{50} values. Higher IC_{50} values indicate low antioxidant activity, that means the lower the IC_{50} the higher the potent the antioxidant property of the extract (11).

Methanol extract showed highest antioxidant activity (84.3%) followed by ethyl acetate extract (82.5%) and n-hexane extract (66.4%) has the lowest activity while the standard oxalic acid showed (94.5%) activity. The extracts showed DPPH radical scavenging activity in a similar manner to that of reference antioxidant oxalic acid decreasing activity with decrease in concentration (Table 1). IC_{50} values were determined by probit analysis using SPSS. IC_{50} is used to express the amount or concentration of extracts needed to scavenge 50% of free radicals. The value of the IC_{50} is inversely proportional to the percentage radical scavenging activity of extracts. For the three extracts n-hexane, ethyl acetate and methanol the IC_{50} values are 17.83µg/ml, 2.21µg/ml and 1.17µg/ml respectively. A study conducted by Yusuf *et al.* (12) showed that methanol extract of *Azanza garckeana* stem bark had better antioxidant activity with IC_{50} value of less than 100µg/ml compared to acetone extract 160µg/ml, (13) also reported that ethyl acetate and methanol extracts of *Azanza garckeana* fruit had strong radical scavenging activity as well as ferric reducing antioxidant power (FRAP). The result of antioxidant assay in this study is in agreement with the report of Yusuf *et al.* (14) and report of Yusuf *et al.* (12).

The antioxidant potentials of the extracts might be associated to phenol and flavonoid in the extracts as reported (14, 15, 16).

Ferric reducing antioxidant power (FRAP) assay method was also adapted to measure the total antioxidant potential of *Azanza garckeana* leaves extracts. The FRAP assay depends upon the reduction of ferric ions to ferrous ions through antioxidant present in the samples. The three extracts (n-hexane, ethyl acetate and methanol) showed similar pattern of increment in their reducing power when the concentrations of the leaves extracts increase. Methanol and ethyl acetate extracts have stronger reducing power than n-hexane extract. Ascorbic acid that was used as positive control showed strongest ferric reducing power.

CONCLUSION

Medicinal plants are playing important roles in traditional medicine. *Azanza garckeana* is one of the important medicinal plants that have great potentials used for the treatment of various diseases. The present study showed that the leaves of Azanza garckeana also have antioxidant potentials and can serve as good source of antioxidant supporting its folkloric

uses in the treatment of diseases. However there is need to study more of the plant in respect to phytochemical content and in vivo assays.

REFERENCES

1. Dasgupta, S. C. (2023). Bioactive Compounds from Medicinal Plants and its Therapeutic Uses in the Traditional Healthcare System. In *Medicinal Plants: Biodiversity, Biotechnology and Conservation* (pp. 525-537). Singapore: Springer Nature Singapore.
2. Vona, R., Pallotta, L., Cappelletti, M., Severi, C., and Matarrese, P. (2021). The Impact of Oxidative Stress in Human Pathology; Focus gastrointestinal Disorder. *Antioxidants*, *10*: 201.
3. Aminjan, H., Abtahia, S.R., Hazrti, E., Chamanara, M., Jalili, M. and Paknejad, B. (2019). Targeting of Oxidative Stress and Inflammation Through ROS/NF-kappaB Pathway in Phosphine-Induced Hepatotoxicity Mitigation. *Life Sciences*, 232, 116607.
4. Pietta, P. G. (2000). Flavonoids as Antioxidants. *Journal Of Natural Products,* 63: 1035-1042.
5. Arefin, P., Shehan Habib, Md., Arefin, A., Saidul Arefin, Md. (2021). Determination of potential sources of drug development for menstrual disorders: A qualitative analysis of published literature of in-vitro rat uterus experimental studies. *International journal of pharmaceutical chemistry and analysis,* (2): 45-48.
6. Maroyi, A. (2017). *Azanza garckeana* Fruit Tree: Phytochemistry, Pharmacology, Nutritional and Primary Healthcare Applications as Herbal Medicine: A Review. *Research Journal of Medicinal Plants, 11*(4): 115-123.
7. Akbar, A., Soekamto, N.H., Firdaus and Bahrun. (2021). Antioxidant of n-hexane, Ethyl acetate and Methanol Extracts of Padina sp with DPPH Method. *International Conference on Sustainable Utilization of Natural Resources, IOP conf. Series: Earth and environmental Science, 800*: 012019.
8. Shah, M. A. R., Khan, R. A., and Ahmed, M. (2019). Phytochemical Analysis, Cytotoxic, Antioxidant and Anti-Diabetic Activities of the Aerial Parts of *Sorghum halepense. Bangladesh Journal of Pharmacology, 14*: 144-151.
9. Shekhar, C.T and Anju G. (2014) Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum Conyzoides Linn.Leaves. *American Journal of Ethnomedicine. 1*(4): 244-249.
10. Vijayalakshmi, M and Ruckmani, K. (2016). Ferric Reducing Antioxidant Power Assay in Plant Extract. *Bangladesh Journal of Pharmacology*, *11*: 570-572.
11. Tufikul, R. H. Mohammad, R. Aumit, R., Shafiqul, I., Afaz, U., Ariful, I., Nuruzzaman, N., and Sohel, R. (2015). "Screening of *In-vitro* Antioxidant, Brine Shrimp Lethality Bioassay and Antimicrobial Activities of Extracts of *Bridelia retusa* (l.) Spreng. Fruit," *International Journal of Pharmacy*, *5*: 1058-1067.
12. Yusuf, A.A., Lawal, B., Sani, S., Garba, R., Muhammed,B.A., Oshevire, D.B., and Adesina, D.A (2020a). Pharmacological Activities of *Azanza garckeana* (Goron Tula) grown in Nigeria'. *Clinical Phytoscience,* 6: 27.

13. Mshelia, E.H., Watirahyel, E.M., Maigari, A.U., Christopher, Y. and Ismail, F. (2016). Cytotoxicity and Antioxidant Activity of Stem Bark Extracts of *Azanza garckeana* (Kola of Tula). *European Journal of Pure and Applied Chemistry. 3*,2.
14. Yusuf, A.A., Garba, R., Alawode, R.A., Adesina, A.D., Oluwajobi, I., Ariyeloye, S.D., Muhammed, I.A., Agboola, R.A., Salisu.L., Abubakar, S., Dan-Smallam, U. and Berinyuy, B.E. (2020b). Effect of Drying Methods and Extractions on Secondary Metabolite Compositions of *Azanza garckeana* Pulp and Shaft. *Noble International Journal of Agriculture and Food Technology, 2*(1): 01-07.
15. Nkafamiya, I. I., Ardo, B. P., Osemeahon,S.A., and Akinterinwa, A. (2016). Evaluation of Nutritional, Non-nutritional, Elemental Content and Amino Acid Profile of *Azanza garckeana* (Goron Tula). *British journal of Applied Science & Technology, 12*(6): 1-10.
16. Michael, K.G., Onyia. L.U., Kefas, M., and Drambi, M. (2015). Cost Benefit of *Azanza garckeana* (Goron Tula) Seed Meal at Different Inclusion Levels in the Diet of *Clarias gariepinus* (Burchell,1822) Juveniles. *Journal of Biology Agriculture and Healthcare.5*,18.