EXTRACTION AND IDENTIFICATION OF REDUCING SUGARS IN *AZANZA GARCKEANA* FRUIT

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ABSTRACT

Azanza garckeana (AG), is a tropical tree widely distributed in Eastern, Southern and Western Africa. The plant species has continued to attract interest due to its cytotoxicity and antioxidant activities. Research on particularly reducing sugars from the plant found in Northern Nigeria remain largely unexploited. Hence, there is a necessity for special research on such compound(s) which could lead to the actual understanding of the medicinal impact of the plant specimen. AG fruit samples were collected from five different areas of Tula town, Gombe state Nigeria and prepared accordingly for sugars analysis. Fehling's solutions A and B, then chromatography analysis was used to test the presence of reducing sugars. Ethanol extraction method of Benedict's solution test was applied for reducing sugar test. Fehling's solutions gave a brick red precipitate indicating the presence of reducing sugars. Orange-brown precipitate was observed from the ethanolic extract solution for reducing sugars test. The compounds extract was found to contain, glucose, mannose, galactose, fructose and sucrose. The functional groups OH, C=O and other absorption bands were identified by IR spectroscopy. Presence of the sugars in the AG fruits confirms its consumption safety and as good source of energy.

Keywords: *Azanza Garckeana* fruit, reducing sugars, isolation, chromatography

INTRODUCTION

Azanza garckeana (AG), a tropical fruit tree is known to be widely distributed in Eastern and Southern Africa (Mbuya *et al* 1994; Mulofwa *et al* (1994) in countries such as Botswana, Zambia, Kenya, Malawi and Mozambique. In Nigeria, it was traditionally believed to be indigenous to Tula in Kaltungo Area (Jacob *et al*, 2016, and Michael *et al*, 2015) but a recent report (Ochokwu and Oshoke, 2014) indicated that it is also found in Michika and Jimeta in Adamawa State. The fact that the plant AG is named Goron Tula (Burkill, 1985) lends credence to the claim that it was first found in that area and probably dispersed to other parts of the country by humans due to its economic value.

The nutritional value of the plant and its characteristics have been widely studied. The plant has continued to attract interest in its cytotoxicity and antioxidant activity (Mshelia *et al* 2016; and phytochemicals (Michael *et al*, 2015) and the proximate analysis of the fruit such as the carbohydrate, ascorbic acid, and some

mineral contents of the fruit have been reported (Jacob *et al* 2016). Abba *et al* (2018) published the taxonomic significance of the plant. The fruit however continues to attract attention as it is claimed to increase fertility, female libido and heightening sexual sensation for women with no side effects. This has demand for the fruit throughout the year round from many parts of the country. But unknown to most people the fruit is seasonal and not available throughout the year. It is however preserved by the local people that grow it. In the process of preservation attracts worms like *Phoenix dactylifera* commonly known as date or date palm fruits. Sequel to that publicity in the social media there has been growing interest by the local people to preserve and grow the fruit on a commercial scale.

In this work we report our findings on the isolation and identification of the reducing sugars in the fruit of AG that should obviously be responsible for the energy content of the fruit that we believe may be linked to the male sexual performance that is attracting wide publicity in the social media. And as far as the authors could find, no report has appeared on the subject. The aim of the study was to provide additional information to our earlier publication (Jacob *et al*, 2016) on the valuable contents of the fruit that has hitherto been lacking.

MATERIALS AND METHODS

Sample collection and preparation

The fruit samples of AG were randomly collected from five different locations in Tula, a hilly terrain in Kaltungo Local Government Area of Gombe State Nigeria. The samples were peeled to remove the seeds while the carpels were later dried in an oven at a temperature of 105^{o}C for about 8-10 hours to remove the moisture content. Some samples were however sun dried. During oven drying sweet aroma was perceived which could probably indicate the presence of some terpenoids.

The dried samples were then crushed using mortar and pestle after which the sweetening substance in the samples were extracted using a literature method described by Kyari (2008)

Tests for simple sugars

Fehling's solutions A&B used to test for the presence of reducing sugars.. Solution A(1ml) and solution B(1ml) were measured and put in a test tube, an aliquot of the extract was added to the Fehling's solution in the test tube and boiled for about ten minutes in a water bath set at 80^{o}C. A brick red colour formation was observed indicating the presence of reducing sugars.

Benedict's test (for reducing sugars)

An ethanol extract was used to test for the presence of reducing sugars. A brick red precipitate indicates the presence of reducing sugar.

Resorcinol (Seliwanoff's test (ketohexoses)

Approximately 0.01g of the reagent grade resorcinol was weighed using analytical balance and dissolved in 20ml of 3molar hydrochloric acid, HCl_{aq} and kept in a dark bottle to protect it from light. Then 1% solution of the ethanol extract was prepared and also 1% solution of the standard sugars (glucose, mannose, fructose, xylose, galactose, ribose, and sucrose) was

prepared. Then 1ml of the resorcinol reagent was measured and placed into 8 separate test tubes and 0.1ml of the sample and standard sugars were added and heated in a water bath at 100^{o}C for about 5minutes and slightly above. The solid sample (i.e. crude) was also tested following the same procedure.

Chromatographic identification of simple sugars

Preparation of colour developing agent

The colour developing agent that was used for both TLC and Paper chromatography was Hydrogen phthalate which was prepared by dissolving with 2.3ml Aniline and 4.0g of phthalic acid in 122.5ml of n-butanol, 122.5 of diethyl ether and 5ml of water and warmed in a water bath at 60^{o}C and kept in a reagent bottle.

Paper chromatography

Paper chromatography was used for the identification of simple sugars in the fruit extract, following standard procedures (Ekpunobi and Eboatu, 2008).:

Preparation of sample: Sample(1ml) was dissolved in distilled water(10ml) and standard sugars(1.0g) were also dissolved in distilled water(10ml) *and* swirled slightly, the resulting solutions were used for sporting both the TLC plate and the chromatographic paper.

Samples were spotted with a capillary tube after a mark on the filter paper at 3cm from the bottom and 3cm in-between each spot using a 30cm ruler and lead pencil for drawing the fainted line at proper position on the paper. The standard sugars that were used include: Glucose, Galactose, Mannose, Fructose, Sucrose, Ribose, and Xylose, they were sported as Gl, Ga, Mn, Fr, Su, Rb, and Xy respectively, while the samples were leballed as Ae and Bw as ethanol and water extract respectively.

Development of chromatogram: The chromatogram was developed by using different solvents systems. The solvent mixture used were n-Butanol, Ethanol and Water (BEW), at the ratio of 4:1:2.; n-Butanol, Acetic acid and Water (BAW) at the ratio of 4: 1: 5; and phenol saturated with water. The sample spotted paper is subjected to development by immersing it in the mobile phase making sure that the sported points doesn't touch the solvent. The mobile phase moves over the sample on the paper under the capillary action of paper.

Drying of the paper and detection of the compounds: Once the development of chromatogram was over, the paper was held carefully at the borders so as to avoid touching the sample spots and then dried using an air drier. The detecting solution (hydrogen phthalate) was sprayed and dried to identify the sample chromatogram spots.

Preparation of the TLC plate: An already prepared Silica gel and aluminum gel TLC plates with the thickness 0.25cm were used for the analysis. The plates were measured to be 20x20 cm; a fainted line of 4cm was drawn from the other end of the TLC plate using a lead pencil.

Preparation of standard sugar and sample solution: The solutions of standard sugars were prepared by dissolving 0.50g of each in 5ml of distilled water and placed into different sample bottles. The same weight of extract was dissolved in 5ml, 8ml

and 10ml of distilled water and properly labeled.

Spotting of the TLC plate: The TLC plate of 0.25cm thickness was carefully spotted using a capillary tube on the marked pencil line at 3cm apart for the standard sugars Glucose, Galactose, Mannose, Fructose, Sucrose, Ribose, and Xylose, which were sported as Gl, Ga, Mn, Fr, Su, Rb, and Xy respectively, while the samples were leballed as Ae and Bw as ethanol and water extract respectively, and allowed to dry.

Development of the chromatogram: the chromatogram was developed by using different solvents system. The solvent mixture used were n-Butanol, Ethanol and Water (BEW), at the ratio of 4:1:2; n-Butanol, Acetic acid and Water (BAW) at the ratio of 4:1:5; phenol saturated with water. But the solvent system that gave separation of sugars on a plain silica gel was mixture of n-butanol, acetic acid, ether and water(BAEW) at the ratio of 9:6:3:1 and 6:4:2:1 then the spotted paper was subjected to development by immersing it in the mobile phase making sure that the spotted points did not touch the solvent. The chromatogram was developed in a TLC tank for about 4-8hrs the mobile phase moves over the sample on the paper under the capillary action. Both the standards and samples were run at the same time with each label as indicated above.

Drying of the chromatograms and detection of the compounds separated: When the development of chromatogram was over, the chromatogram was carefully held at the borders so as to avoid touching the sample spots while the solvent front was marked with a pencil and dried using an air drier and then sprayed with a colour developer (Hydrogen Phthalate) which was again dried in an oven at 105^{o}C for about 10minutes

R_f values: After developing the chromatogram, the colour visualization of the solute front was marked and measured for both the standards and sample. The R_f value was calculated for all the chromatograms.

Infra-red spectra

The infra-red spectrum of the fruit extracts was acquired from NARICT Zaria using a model SHIMADZU FTIR-8400S Fourier Transform Infrared Spectrometer.

RESULTS

The qualitative analyses of the sugars are presented under Table 1. Fehling's solutions A and B gave a brick red precipitate indicating the presence of reducing sugars. From the results of qualitative analysis, it can be seen that both the ethanol and the water extracts of the samples also gave a positive test for reducing sugars using Benedict's solution i.e. an orange-brown precipitate was observed.

Table1: Qualitative tests for reducing sugars

Sugars	Fehling Solution A & B	Benedicts Solution	Resorcinol	Iodine
Glucose	Positive	positive	Negative	Negative
Mannose	Positive	positive	Negative	negative
Galactose	Positive	positive	Negative	negative
Ribose	Positive	positive	Negative	negative
Xylose	Positive	positive	Negative	negative
Sucrose	Negative	negative	Positive	negative
Fructose	Positive	positive	Positive	negative
Sample Ae	Positive	positive	Positive	negative
Sample Bw	Positive	positive	Positive	negative

Table 2 shows the separation of the sugars obtained by paper chromatography. The solvents used were n-butanol/acetic acid/water (BAW); n-butanol/ethanol/water (BEW); n-butanol/acetic acid/ether/water (BAEW); and phenol saturated with water (PHOL). The results obtained showed two distinct spots, Ae and Bw, both having brown colours. The use of BEW as solvents system for elution in the paper chromatography gave average (Av.) R_f value of 21.0 for Ae and 22.0 for Bw. These R_f values can be compared with those of glucose and galactose with the R_f values of 21.5 and 22.0 respectively which were run at the same time.

Table 2: R_f values, colours and solvents for paper chromatography

Sugar	**BEW**	**R_f x100**		**BAW**	**R_f x100**		**PHOL**	**R_f x100**		
	1st	**2nd**	**Av.**	**1st**	**2nd**	**Av.**	**1st**	**2nd**	**Av.**	**Colour with Aniline hydrogen phthalate**
Glucose	21.0	22.0	**21.5**	64.0	64.0	**64.0**	97.2	97.0	**97.1**	Brown
Galactose	22.0	22.0	**22.0**	67.0	67.0	**67.0**	97.6	97.4	**97.5**	Brown
Xylose	39.0	39.0	**39.0**	72.0	73.0	**72.5**	99.2	99.2	**99.2**	Red
Ribose	40.0	42.0	**41.0**	73.0	73.0	**73.0**	97.6	97.6	**97.6**	Red
Mannose	34.0	34.0	**34.0**	80.0	78.0	**79.0**	96.0	96.2	**96.1**	Brown
Sample A_e	21.0	21.0	**21.0**	68.0	68.0	**68.0**	97.2	97.2	**97.2**	Brown
Sample B_w	22.0	22.0	**22.0**	67.0	67.0	**67.0**	97.4	97.4	**97.4**	Brown

Key notes: *BEW= n-Butanol-Etha nol-Water (4:1:2)*
BAW= n-Butanol- Acetic acid- Water (4:1:5)
PHOL= Phenol saturated with water
Av. = Average

On the other hand, when BAW was used as the mobile phase, the R_f values for the sample spots Ae and Bw, were 68.0 and 67.0 respectively. These compare favaourably well with those of glucose and galactose with R_f values of 64.0 and 67.0 respectively. The fact that different R_f values were obtained for the same substance for different solvents is expected since the values usually depend on the solvent used. Another solvent system used, phenol (PHOL), gave high R_f values for the

standard samples when compared with those obtained with other solvents. The phenol extract appears to have high R_f values probably because the more polar the solvent the more its eluting power. The values are however in close agreement with those of fruit extracts of 97.2 and 97.4 for Ae and Bw respectively. These values tally with those for glucose and galactose with the R_f values of 97.1 and 97.5 respectively.

Further separation of the extracts, Ae and Bw, by thin layer chromatography (TLC), using two different solvents systems, BEW and BEAW, are presented in Table 3. The chromatogram obtained with BEW also gave two spots with R_f values of 54.2 and 54.2 respectively which is comparable to our standard glucose and galactose of 52.2 and 54.3 respectively. But with BAEW as eluting solvents systems, the R_f value for Ae and Bw were both 33.0 which can be seen to exhibit the same characteristics as glucose, galactose and fructose having the close R_f values of 34.5. When standard sucrose was run on the TLC plate with BEW as solvent there was no visible spot. However a brown colour was observed with the BAEW solvent and with R_f value of 10.5.

Table 3: R_f values, colours and solvents for TLC

Sugar	BEW	R_f x100		BAEW	R_f x100		Colour with Aniline hydrogen phthalate
	1st	**2nd**	**Av.**	**1st**	**2nd**	**Av.**	
Glucose	54.3	54.2	**54.25**	35.0	34.0	**34.5**	Brown
Galactose	54.3	54.3	**54.30**	35.0	33.0	**34.0**	Brown
Xylose	70.9	70.8	**70.75**	52.0	52.0	**52.0**	Red
Ribose	70.9	70.9	**90.90**	45.0	45.0	**45.0**	Red
Mannose	60.6	60.5	**60.55**	38.0	33.0	**35.5**	Brown
Fructose	-	-	-	35.0	34.0	**34.5**	Brown
Sucrose	-	-	-	11	10	**10.5**	Brown
Sample A_e	54.3	54.0	**54.15**	33.0	33.0	**33.0**	Brown
Sample B_w	52.2	52.2	**52.2**	35.0	33.0	**34.0**	Brown

Key: BEW= n-Butanol - Ethanol – Water (4:1:2). BAEW= n-Butanol – Acetic acid – Ether – Water (9:6:3:1), Av. = Average

It appears the solvent systems that gives good separation on the TLC plate was BAEW and BEW for paper chromatography. A chromatogram of TLC with BAEW as solvent system is given under Figure 1.

Figure 1: Ripened fruits of *Azanza garckeana*

The infrared spectrum of the fruit extract is shown in Figure 2, while absorption frequencies are tabulated in Table 4. The absorption frequencies at 3431cm^{-1}, 2924cm^{-1}, 1721cm^{-1}, 1651cm^{-1}, and 1403cm^{-1} could be assigned to the stretching vibrations corresponding to the functional groups O-H, C-H, C=O, C-C and C-O respectively. The rest of the peaks are in the finger print region which is not so useful for the functional group identifications. The samples had sweet flavours like that of honey. So, the absorption band around 1700cm^{-1} assigned to the C=O stretching vibration could be from an ester since these compounds are known to possess this kind of aroma. The extracts were syrups. Hence the broad peak around 3400cm^{-1} is usually characteristic of water since the extract was not totally free from water and our attempts to crystallize the syrup were unsuccessful.

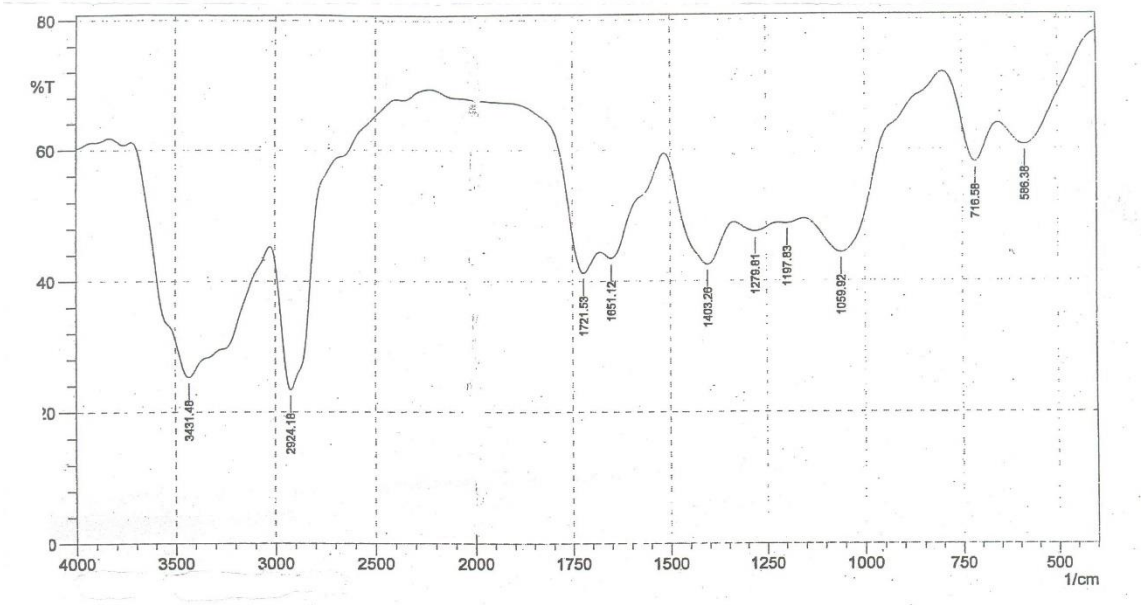

Figure 2: Infra-red Spectrum of *Azanza garckeana* fruit extract.

Table4: IR Absorption frequencies and the assigned functional groups

Frequency(cm^{-1})	Stretching vibration
3431	O-H
2924	C-H
1721	C=O
1651	C=C
1403	C-O

DISCUSSION

The test with Resorcinol reagent involves the hydrolysis of sucrose to two units i.e. glucose and fructose and it is the latter that then reacts with the reagent to give a brick red colour. Also, from Table 1, Resorcinol reagent (Seliwanoff's test) gave brick red colour with the standard samples (sucrose and fructose) for reducing sugars. The fact that a positive test was obtained with the standard samples and the fruit extracts indicated the presence of sucrose and or fructose. The fructose was either present in the sample initially or it was from the hydrolysis of sucrose during the extraction process. The resulting positive test for reducing sugars obtained with standard glucose and a negative test with resorcinol does not rule out the presence of glucose in the fruit extract since resorcinol is used to

distinguish glucose from fructose. Iodine solution gave a negative result for the standards and the fruit extracts, which indicates the absence of starch. From the results of the qualitative tests it is apparent that the extracts contain reducing sugars rather than starch.

From these results, it could be inferred that the fruit contains glucose and galactose though the former could be a product of hydrolysis from sucrose. However, the average R_f values 22 obtained with BEW as the mobile phase, could mean the presence of glucose or galactose or both since their R_f values are very close (21.5 and 22) as compared with our standard samples. The R_f values obtained agrees with literature (Harbone, 1969) though slightly higher by 5 units but agrees fairly well with a value of 20 reported by Ekpunobi and Eboatu (2008) for glucose. From the findings of this study, the isolation of the sugars in the AG fruit, by paper and thin layer chromatography showed the presence of simple sugars that is glucose, galactose and fructose. However, the qualitative tests indicated the presence of sucrose. This could mean that the sucrose was probably hydrolysed as explained earlier.

The IR technique employed confirmed only the presence of some functional groups and was not therefore informative about the different types of sugars present since all the sugars basically contain these functional groups. Furthermore, the importance of sugars in the body is well known especially glucose which is the source of energy for all cellular activities in the body including the brain and nervous system.

CONCLUSION

The identification of simple sugars in the fruits of AG was done successfully using qualitative tests and separation by chromatographic techniques (paper and thin layer chromatography). From the findings of this study, the fruits contain a mixture of reducing sugars such as glucose, galactose, fructose (monosaccharide) and sucrose as a disaccharide. But whether the simple sugars are originally present or as products of the hydrolysis of sucrose could not be ascertained. However, the presence of the sugars in the fruit confirms its consumption as a good source of energy.

REFERENCES

Abba, H.M, Sale, S. and D. A. Zhigila (2018). Taxonomic Significance of Foliar Epidermal Characters in Azanza garckeana (F. Hoffin) Excell & Hillc in Tula, Kaltungo Local Government Area of Gombe State, Nigeria. American Journal of Plant Sciences Vol. 9. Pp 1677 – 1687.

Burkill, H. M (1985). The Useful plants of West Africa, Vol. 4. Fundamentals of Analytical Chemistry 8th ed. Cengage Learning Indian Private limited, India.

Ekpunobi E.U and Eboatu A.N (2008), Extraction of Sugar from Nypa Palm (Nypa nnnnn fruitican) Journal of Chemical Society of Nigeria, 33 (1) pp122-125.

Mbuya L. P., Msaga C.K, Ruffo, Birnie A., Tengas B. (1994). Useful trees of Tanzania Regional Soil Conservation Unit/ SIDA. 23-27 (2).

Michael K. G, Onyia, L. U, and Jidauna, S. B. (2015). Evaluation of Phytochemicals in *Azanza garckeana* (Goron Tula) Seed. Journal of Agriculture and Veterinary Sciences Volume 8 Issue 5 pp 71-74.

Mshelia, E. H; Watirahyel, E. M.; Maigari, A. U; Yohanna Christopher, and Ismail, F. Cytoyoxicity and Antioxidant Activity of Stem Bark Extracts of *Azanza garckeana* (kola of Tula). European Journal of Pure and Applied Chemistry (2016) Vol. 3. pp16 – 24.

Mulofwa, J. S; Simute, S; Tungas, B. (1994) Agroforestry Manual for Extension Workers in Southern Province Zambia. Technical Book No. 4. Regional; Soil Conservation Unit/SIDA.

Ochokwu, I. J; Dasuki, A.; and Oshoke, J. O. (2015). Azanza garckeana (goron Tula) as an Edible Indigenous Fruit in North eastern Part of Nigeria. Journal of Biology, Agriculture and Healthcare. Vol, 5, No, 15, pp 26 – 31.