



Research Article

Phytochemical analysis of Hexane, Chloroform, Ethyl acetate, Ethanol and Aqueous Extracts of *Azanza garckeana* Leaf

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ABSTRACT

The study evaluated the qualitative phytochemical content of different extracts of *Azanza garckeana* leaf. Phenols, carbohydrates, alkaloids, saponins, steroids, terpenes, tannins, cardiac glycosides and flavonoid contents were evaluated using standard methods. The results showed that tests for steroids, terpenes and carbohydrates were positive for all the extracts. Cardiac glycosides was found to be present in all the extracts except for hexane and aqueous extracts. Phenols were only found in the aqueous, ethanol and ethyl acetate extracts. Saponins were present in the aqueous and ethanol extracts while flavonoids, tannins and alkaloids were only found in the aqueous extracts. The presence of these phytoconstituents may give credence to its medicinal uses.

Keywords: Phytochemicals; *Azanza garckeana*; Alkaloids; Saponins; Flavonoids

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INTRODUCTION

Plants have been used as sources of medicine throughout the world to preserve human health and are rich source of components with a variety of biological activities. The secondary metabolites contained in plants are responsible for their medicinal activities. Approximately 95% of modern drugs have been isolated from traditional medicinal plants [Masoko, 2017]. Plants are traditionally used in the form of infusions, decoctions, tinctures or herbal extracts, in some cases, they are used in combination with 2 or more plants for treatment of a particular disease [Wendakoon *et al.*, 2012]. Different plant' parts have been used for treatment of various form of ailment. Medicinal plants play a vital function in both development and production of new drugs [Sofowora *et al.*, 2013]. The

investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades because of their potent pharmacological activities [Rahman and Islam, 2013].

Chemical substances are found in several medicinal plants that are known as phytochemicals which have been shown to possess therapeutic potentials that could be harnessed for curative purpose [Momodu *et al.*, 2021]. The identification of these phytochemical via phytochemical screening paves way for the isolation and characterization of novel compounds with promising biological potentials [Obayuwana *et al.*, 2020].

Azanza garckeana (*A. garckeana*) belonging to Malvaceae family has been selected for this work. The plant *Azanza garckeana*, commonly known as Goron Tula, (kola of Tula) in Hausa [Burkill, 1985],

belong to the family Malvaceae, in the order Malvales. *A. garckeana* belong to a family malvacene, the distriptive family to which the hibiscus belongs. Most of the malvacene in Nigeria are shrubs. The strip soil desert coast extending below the equator in Africa was once known as the course of Azanza. The name Azanza being based on a word meaning black and survival in Zanzibar and it is possible that Azanza is derived from this. The specific name *garckeana* was given after Professor August Garcke (1891 – 1904), a German botanist [Orwa *et al.*, 2009].

The bark, roots, fruits, leaves and stems of *A. garckeana* are reported to possess a wide range medicinal properties and used to treat or manage various diseases and ailments throughout its distributional range [Maroyi, 2017]. Total of twenty-two (22) traditional medicinal uses of *A. garckeana* are documented in literature from 9 countries in tropical Africa, representing 64.3% of the countries where the species is indigenous [Mshelia *et al.*, 2016]. In the DRC, the leaf or root decoction of *A. garckeana* is taken orally as remedy for diabetes, edema, and epilepsy and membrane rupture [Amuri *et al.*, 2017; Esther *et al.*, 2017]. A decoction is made from the roots are taken orally for management of painful menstruation and also used to treat coughs and chest pains. An infusion made from the roots and leaves is dropped into the ear to treat earache or taken orally as an antiemetic [Maroyi, 2013]. Thus, main aim of this research work is to analyze the phytochemical content of Hexane, Chloroform, Ethyl acetate, Ethanol and Aqueous extracts of *Azanza garckeana* leaf.

MATERIALS AND METHODS

Plant Materials

The leaves of the plant, *Azanza garckeana* of the family of Malvaceae were procured from Tula, Kaltungo LGA of Gombe State. They were identified at the Biological sciences department, Bayero University Kano, Kano state, Nigeria. And the voucher number BUKHAN 650 and was deposited for future reference.

Preparation of Plant Materials

Azanza garckeana leaves procured were dried under shade for 5 days. They were pulverized into powder form using an electric blender.

Serial extraction

1.2 kg of the powdered sample was packed into glass bottles and was extracted successively using, n-

hexane, chloroform, Ethyl acetate, Ethanol and water (maceration). At the end of the extraction, the extracts obtained were concentrated at 40°C using a rotatory evaporator, dried to a constant weight in a pre-weighed petri-dish and then kept in a refrigerator before subsequent analyses.

Chemicals/Reagents

All chemicals and reagents used were of analytical grade. Molisch reagents, Mayer's reagents, Distilled water, ethyl acetate, ethanol, hexane and chloroform were obtained from E. Merck (Germany). All the reagents were used without any further purification.

Phytochemical Screening

Phytochemical screening to identify the phytochemical constituents of the plant using standard methods described by (Evans, 2009; Sofowora, 2008).

Test for Carbohydrates

(i) Molisch's Test (General Test): 0.5 g of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was treated with 2 drops of Molisch's reagent in a test tube, followed by 1 ml of concentrated H₂SO₄ down the side of the test tube. Formation of reddish colored ring at the junction indicates the presence of carbohydrates (Evans, 2009).

(ii) Fehling's Test (Reducing Sugar): 0.5 g of the extract was dissolved in 5 ml of distilled water and filtered. The filtrates were hydrolyzed with dilute HCl, neutralized with alkali and heated with mixture of equal volume of Fehling's A & B solutions. Formation of brick red precipitate indicates the presence of combined reducing sugars (Evans, 2009).

Test for Cardiac Glycosides

(i) Keller-killiani test: The extract (0.1 g) was dissolved in 2ml glacial acetic acid containing traces of ferric chloride solution. This was then transferred in to a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Appearance of a purple brown ring at the interphase indicates the presence of deoxy sugars and a pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

(ii) Kedde test: 0.1 g of the extract was treated with 1ml of 2% solution of 3, 5- dinitrobenzoic acid in 95% alcohol. The solution was made alkaline by the

addition of 5% NaOH. The presence of purple- blue color indicates the presence of cardenolides (Evans, 2009).

Tests for Saponins

(i) Frothing test: About 0.5g of the extract was dissolved in 10 ml of water and shaken vigorously for 30 seconds and allowed to stand for one hour, the occurrence of frothing column or honey comb-like of at least 1 cm in height and persisting for at least 30 minutes indicates the presence of saponins (Sofowora, 2008).

(ii) Haemolysis test: 2 ml of sodium chloride (1.8% solution in distilled water) was added to two test tubes A and B. 2 ml of distilled water were added to test tube A, 2 ml of the extract was added to test tube B. 5 drops of blood was added to each tube and the tubes were inverted gently to mix the contents. Haemolysis in tube B containing the extract but not in tube A (i.e., control), indicate the presence of saponins in the extract (Evans, 2009).

Test for Flavonoids

(i) Shinoda test: 0.1 g of the extract was dissolved in 2 ml of 50% methanol in the heat metallic magnesium fillings and 2 drops of concentrated hydrochloric acid were added and a pink, rose or red coloration indicated the presence of flavonoids (Evans, 2009).

(ii) Sodium hydroxide test: 0.1 g of the extract was dissolved in water and filtered; 2 ml of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid was used as an indication for the presence of flavonoids (Evans, 2009).

Test for Steroids/ Triterpenes

(i) Lieberman-Burchard test: 1 ml of acetic anhydride was added to 0.5 g of the extract. Two ml of concentrated sulphuric acid was then added gently by the side of the test tube to form lower layer and at the junction of the two liquids formation of reddish brown or violet brown ring, the upper layer bluish green or violet indicates the presence of sterols and triterpenes. (Evans, 2009).

(ii) Salkowski test: 2 ml of chloroform was added to 0.5 g of the extract and two ml of concentrated sulphuric acid was carefully added from the side of the test tube to form a lower layer. A reddish-brown

coloration at the interface indicated the presence of steroidal ring (Sofowora, 2008).

Test for Tannins

(i) Ferric chloride test: 0.5g of the extract was dissolved in 5ml of water and filtered. Two drops of ferric chloride solution were added to the filtrate. Appearance of blue-black or green or blue-green (condensed tannins) precipitate indicates the presence of tannins (Evans, 2009).

(ii) Lead sub-acetate test: To a small quantity of the extract, two drops of lead sub acetate solution were added and appearance of whitish-yellow precipitate indicates the presence of tannins. (Evans, 2009).

Test for Alkaloids

About 1.0 g of extract was stirred with 20ml of 1% aqueous hydrochloric acid on a water bath and filtered. The filtrate was basified with concentrated NH₄OH and extracted with chloroform. The chloroform layer was then extracted with 20ml of 1% HCl. The aqueous layer was divided into three portions for the following tests:

- To the first portion, 1ml of freshly prepared Dragendorff's reagent was added drop-wise and observed.
- To the second portion 1ml of Mayer's reagent was added drop-wise and observed.
- To the third, 1 ml of Wagner's reagent was also added.

Appearance of rose red to brownish, white to yellowish or cream color and brown or reddish-brown precipitates respectively indicate the presence of alkaloids (Evans, 2009).

Test for Phenols (Ferric Chloride Test)

The extract was dissolved in alcoholic solution. Exactly 1 mL of distilled water was placed in to 5 mL of extract alcoholic solution, followed by drops of 10% aqueous ferric chloride (FeCl₃) solution. The presence of phenols was demonstrated by the production of a blue coloration (Evans, 2009).

Data Analysis

The data obtained are presented in Table with + = presence of phytochemical and - = absence of phytochemical.

RESULTS

The phytochemicals present in the various extracts of *Azanza garckeana* are shown in Table 1. The results showed that steroids, terpenes and carbohydrates were positive for all the extracts. Cardiac glycosides was found to be present in all the extracts except for hexane and aqueous extracts.

Phenols were only found in the aqueous, ethanol and ethyl acetate extracts. Saponins were present in the aqueous and ethanol extracts while flavonoids, tannins and alkaloids were only found in the aqueous extracts.

Table 1. Phytochemical content of Hexane, Chloroform, Ethyl acetate, Ethanol and Aqueous extracts of *Azanza garckeana* leaf

Test (phytochemicals)	Hexane Extracts	Chloroform Extracts	Ethyl acetate Extracts	Ethanol Extracts	Aqueous Extracts
Saponins	-	-	-	+	+
Terpenes	+	+	+	+	+
Phenols	-	-	+	+	+
Flavonoids	-	-	-	-	+
Tannins	-	-	-	-	+
Cardiac glycosides	-	+	+	+	-
Carbohydrate	-	+	+	+	+
Steroids	+	+	+	+	+
Alkaloids	-	-	-	-	+

+ = Present; - = Absent

DISCUSSION

Extraction of phytochemical compounds from medicinal plants is highly dependent on the type and polarity of solvent used [Masoko *et al.*, 2008]. Different solvents possess different extraction abilities, which are dependent on both the polarity of the extraction medium and the different types of solvents used [Allothman *et al.*, 2009]. Phytochemicals are referred to as non-nutrient plant component that are found in various plant parts such as its fruits, leaves, stem and roots [Momodu, *et al.*, 2021]. The consumption of medicinal plants with certain phytochemicals has been found to be related with a decline in the risk of several major chronic diseases [Saxena *et al.*, 2013]. The phytochemical composition of the different extracts of AG leaf is presented in Table 1. The results indicate significant variation in the contents of the different phytochemicals between the five different solvents used in this study: water, ethanol, ethyl-acetate, chloroform and hexane. The aqueous extracts of the plant had significantly higher content of phytochemicals tested as it contained all those

tested for which include saponins, terpenes, phenols, flavonoids, tannins, carbohydrate, steroids, and alkaloids however, it did not test positive for Cardiac glycosides. The ethanol extract had the next highest yield as it tested positive for all the above mentioned phytochemicals except for flavonoids, tannins and alkaloids. The ethyl-acetate extract tested positive for terpenes, phenols, cardiac glycosides, carbohydrates and steroids. The chloroform extract tested positive for terpenes, cardiac glycosides, carbohydrates and steroids. While the hexane extracts tested positive for only terpenes and steroids. This findings correlates with the findings of Momodu *et al.* [2021] on the phytochemical screening of the pulp of *A. garckeana*.

The differences in the extracting abilities may be related to the differences in polarity of the components extracted and that of the solvents [Akinrinde *et al.*, 2018]. Alkaloids and saponins are relevant for their roles as analgesic and antispasmodics. Saponins possess a wide range of pharmacological activities, including anti-

inflammatory, vasoprotective, gastro protective properties [Abifarin *et al.*, 2019], they also have been documented to be antifungal, antibacterial, and antioxidant agents [Aluko *et al.*, 2012]. Total phenols and flavonoids possess modulatory effects against lipid peroxidation involved in atherogenesis and carcinogenesis [Mbaebie *et al.*, 2012]. Phenolic compounds are known to possess antibacterial action [Ofokansi *et al.*, 2005]. Flavonoids have antioxidant effects and have been shown to inhibit the initiation, promotion, and progression of tumours and also reduce the progression of coronary heart disease. Other biological functions of flavonoids include protection against platelet aggregation, hepatotoxins, viruses, tumours, ulcers, free radicals, inflammation, and allergies. Phenols are antioxidants in human and plants [Ezeonu and Ejikeme, 2016]. Tannin a polyphenol possesses various medicinal, therapeutic properties and exhibits various pharmacological properties such as anti-cancer, anti-allergic and anti-inflammatory, anthelmintic, antimicrobial, antiviral, healing of wounds, curing of dysentery (Sharma *et al.*, 2021). Moreover, tannin has been found to enhance glucose uptake, leading to a reduction in blood sugar levels and a decreased risk of diabetes [Kumari and Jain, 2015]. It has been documented to selectively inhibit HIV replication [Ezeonu and Ejikeme, 2016]. Cardiac glycosides (CGs) have been suggested to possess promising anticancer properties through various mechanisms of action [Guerero *et al.*, 2019]. The most important use of the cardiac glycosides is its effects in treatment of cardiac failure, they inhibit Na⁺, K⁺-ATPase, and consequently increase the force of myocardial contraction. Some cardiac glycosides were investigated for their antitumor activity. In addition, it has been reported that some cardiac glycosides display an inhibitory activity against rhinovirus [Morsy, 2017]. A growing body of research has unveiled the selective anti-proliferative and pro-apoptotic effects of cardiac glycosides on various human cancer cell lines, including those associated with cervical, lymphoma, lung, colon, breast, prostate, melanoma, pancreas, and liver cancers. Consequently, several cardiac glycosides have been subjected to clinical trials as potential anticancer agents [Yang *et al.*, 2022]. Polysaccharide have been found to act as antidiabetic agents through multiple mechanisms, including the augmentation of serum insulin levels, the reduction of blood glucose levels, and the enhancement of glucose tolerance. Numerous polysaccharides have also been scientifically proven to be advantageous in the treatment of hypoglycemia [Gaikwad *et al.*, 2014]. They also exhibit immuno-modulatory activity

in vitro. Other actions include an antimicrobial action against *Streptococcus mutans*, induction of cell differentiation, inhibition of angiogenesis and an antimetastatic effect. The effectiveness of polysaccharides varies significantly, with their greater complexity in branched chains and higher molecular weight being directly proportional to their increased bioactivity [Teoh, 2016]. Finally, steroids are bioactive compounds that are present naturally in many plants and known to possess potent hypoglycaemic activity [Gaikwad *et al.*, 2014]. They are also known to possess anticancer properties through interaction with various cell targets and pathways, antioxidant, anti-inflammatory, antieryptotic, antihaemolytic effects, neuroprotective and even microbiota modulatory abilities (Salehi *et al.*, 2021).

In conclusion, this study shows that the leaf of *A. garckeana* contains significant bioactive phytochemicals which could support its use in ethno medicine as well as serve as a scientific proof for the traditional usage of this plant for the treatment of diseases.

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REFERENCES

Abifarin, T. O., Afolayan, A.J. and Otunola, G. A. (2019). Phytochemical and antioxidative activities of *Curcumis africanus* L.f. A wild vegetable of South Africa. *Journal of Evidence-Based Integrative Medicine*. 24:1-8.sagepub.com/journals-permissions DOI: 10.1177/2515690X19836391.

Akinrinde A.S., Afolayan A.J. and Bradley G. (2018) Phytochemical composition and antioxidant activities of *Dianthus Thunbergii* hooper and *Hypoxis Argentea* harv ex baker: Plants used for the management of diabetes mellitus in Eastern Cape, South Africa. *Pharmacognosy Magazine*. 14:195-202.

- Allothman M, Bhat R. and Karim AA. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*. 115:785-788.
- Aluko, B.T. Oloyede, O.I. and Afolayan A.J. (2012). Phytochemical and nutrient compositions of the leaves of *Ocimum canum* Sims. *African Journal of Biotechnology*. 11(63): 12697 – 12701.
- Amuri, B., Maseho, M., Simbi, L., Okusa, P., Duez, P. and Byanga, K. (2017). Hypoglycemic and antihyperglycaemic activities of nine medicinal herbs used as antidiabetic in the region of Lubumbashi (DR Congo). *Phytotherapy Research*. 31: 1029-1033.
- Burkill, H. M. (1985). The useful plants of West Tropical Africa. Vol. 2. Royal Botanic Gardens. U.K.
- Esther, K.I., Kleph, K.Z., Mariette, K.K, Sylvie, Y.N. and Andre, W.O.T. (2017). Plants used by pregnant women at Kipushi city in democratic republic of Congo: Prevalence and indications. *Open Access Library Journal*.4:3390. <https://doi.org/10.4236/oalib.1103390>.
- Evans, W. F. (2009). Trease and Evans Pharmacognosy. 16th ed. Elsevier Saunders, London: p603.
- Ezeonu, C.S. and Ejikeme, C. M. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. Hindawi Publishing Corporation. *New Journal of Science*. 2016:5601327, 9 pages. <http://dx.doi.org/10.1155/2016/5601327>
- Gaikwad, B. S., Mohan, K. G. and Rani, M.S. (2014). Phytochemicals for Diabetes Management. *Pharmaceutical Crops*. 5(1):11-28. 2014 Bentham Open.
- Guererro, A., Herranz, N., Sun, B., Wagner, B., Gallage, S., Guiho, R., et al. (2019). Cardiac glycosides are broad spectrum senolytics. *Nature Metabolism*.1:1074-1088. <https://doi.org/10.1038/s42255-019-0122-z>
- Kumari, M. and Jain, S. (2015). Screening of potential sources of tannin and its therapeutic application. *International Journal of Nutrition and Food Sciences*. 4 (2): 26–29.
- Maroyi, A., (2013). Traditional use of medicinal plants in South-central Zimbabwe: Review and perspectives. *Journal of Ethnobiology and Ethnomedicine*. 9(1):31. 10.1186/1746-4269-9-31.
- 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: 115-123.
- Masoko, P. (2017) Phytochemical Analysis, Antioxidant and Antibacterial Properties of *Spilanthes mauritiana* Used Traditionally in Limpopo Province, South Africa. *Journal of Evidence-Based Complementary & Alternative Medicine*. 22(4) 936-943. sagepub.com/journalsPermissions.nav. DOI: 10.1177/2515690X17746774.
- Masoko P., Mokgotho, M.P., Mbazima, V.G. and Mampuru, L.J. (2008). Biological activity of *Typha capensis* (Typhaceae) from Limpopo Province (South Africa). *African Journal of Biotechnology*. 20:3743-3748.
- Mbaebie, B. O., Edeoga, H. O. and Afolayan A. J. (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*. 2012: 118-124.
- Momodu, I.B., Agoreyo, B.O., Okungbowa, E.S., Igiebor, S. E. and Igbo, I.O.L. (2021). Phytochemical Screening and Proximate Composition of Aqueous-Methanol Pulp Extract of *Azanza garckeana* (Goron Tula). *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, 7(4a). <https://dx.doi.org/10.4314/dujopas.v7i4a.20> ISSN (Print): 2476-8316 ISSN (Online): 2635-3490.
- Morsy, N. (2017). Cardiac Glycosides in Medicinal Plants. Aromatic and Medicinal Plants - Back to Nature. Chapter2: 30-45. Intechopen. <http://dx.doi.org/10.5772/65963>.
- Mshelia, E.H., Watirahyel, E.M., Maigari, A.U., Yohanna, C. 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: 16-24.
- Obayuwana, O., Imafidon, K. E. and Abu, O. D. (2020). Phytochemical and proximate composition of leaves of *Anarcadium occidental*. *IAR Journal Agriculture Research Life Sciences*. 1(5): 139-142.

Ofokansi, K. C., Esinone, C. O. and Anele, C. K. (2005). Evaluation of the leaf extracts of *Bryophyllum pinnatum* (Fam. Crassulaceae) and *Ocimum gratissimum* (Fam.Labiatae). *Plant Production Research Journal*. 9: 23 – 27.

activities. *RSC Advances*. 12:23240. DOI: 10.1039/d2ra04464a.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. (2009). *Azanza garckeana* (F. Hoffm.) Exell ET Hill. Agro forestry Database Tree Reference and Selection Guide Version 4.0. http://www.worldagroforestry.org/treedb/AFTPDFS/Azanza_garckeana.PDF.

Rahman, M. A. and Islam, M. S. (2013). Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract. *Asian Pacific Journal of Tropical Biomedicine*. 3(4):273-279. Doi: 10.1016/S2221-169191(13)60062-3

Salehi, B., Quispe, C., Sharifi-Rad, J., Cruz-Martins, N., Nigam, M., Mishra, A. P., *et al.*, (2021) Phytosterols: From Preclinical Evidence to Potential Clinical Applications. *Frontiers in Pharmacology*. 11:599959. doi: 10.3389/fphar.2020.599959.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*. 1(6):169-182.

Sofowora, A., Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines*. 10:5. DOI: 10.4314/ajtcam.v10i5.2

Sofowora, A.A. (2008). Medicinal plants and traditional medicine in West Africa. 3rd Ed. John Wiley and Sons Ltd. New York pp. 200-203.

Teoh, E. S. (2016). Secondary metabolites of plants. *Medicinal Orchids of Asia*. p. 59-73 DOI 10.1007/978-3-319-24274-3_5. Springer International Publishing Switzerland.

Wendakoon C, Calderon P, Gagnon D. (2012). Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *Journal of Med Active Plants*. 1:60-68.

Yang, H-Y., Chen, Y-X., Luo, S., He, Y-L., Feng, W-J., Sun, Y., Chen, J. and Gao, K. (2022). Cardiac glycosides from *Digitalis lanata* and their cytotoxic