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Evaluation of the phytochemical/active ingredients composition of the hydroethanol extract of the fruit pulp of *Azanza garckeana* and its toxicity profile

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Abstract
Prior to in depth explorations of therapeutic potentials of medicinal plants and products, the focus should be on detailed screening and identification of their active ingredients. Consequently the present study evaluated the phytochemical/active ingredients composition of the hydroethanol extract of the fruit pulp of *Azanza garckeana* (Az-garck*)* and its toxicity profile. The plant belongs to the Malvaceae family and is commonly called Gorontula in Nigeria and snot apple/wild hibiscus in English. The plant is said to possess numerous dietary and medicinal attributes. Crude extraction of the plant's fruit pulp using hydro-ethanol (30:70 v/v) as solvent was done. The phytochemical properties of the plant extract was ascertained by qualitative phytochemical screening using standard test methods. Further, the gas chromatography and mass spectrometry (GCMS) analysis of the extract was also done using GC Clarius 500 Perkin Elmer analyzer. And lastly, the acute toxicity screening of the Az-garck fruit pulp was determined using the Lorke's Method. The results of the aforementioned screenings revealed semi-solid concentrated crude extract of the plant portion; secondary metabolites such as alkaloids, carbohydrates, steroids/triterpene as phytochemicals. The GCMS analysis of Az-garck, identified five (5) active compounds namely Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl; hexadecanoic acid, ethyl ester (Palmitic acid); 9, 12-Octadecadienoic acid, ethyl ester (Ricinoleic acid) and octadecanoic acid, ethyl ester (methyl stearate). Meanwhile, the LD_{50} of the extract was reported to be more than 2000mg/kg bw for Az-garck fruit pulp. Finally, the present study has shown that the hydroethanolic fruit pulp extract of Az-garck possess rich active compounds with probable numerous therapeutic and beneficial effects and these may be the rationale behind its wide ethnobotanic applications in our locale and beyond.

Keywords: Hydroethanol extract, fruit pulp of *Azanza garckeana*, GCMS analysis, toxicity profile, phytochemical constituents

Introduction
Azanza garckeana (Az-garck) is a specie of the Malvaceae family. It is commonly called Gorontula in Nigeria, Morojwa in Botswana, *Thespesia garckeana* in South Africa and Snot Apple, Wild Hibiscus and African Chewing gum in English [1]. The plant is predominant in Nigeria, Congo, Sudan, Kenya, Namibia, Tanzania, Zambia, Zimbabwe, Mozambique, Malawi, South Africa, Burundi and Botswna [2-4]. In Nigeria, it is mainly cultivated in Kaltungo Local Government Area of Gombe State and in Kali Hills, Zah, Michika Local Government Area, Adamawa State [5, 1].

Goron Tula is a deciduous small tree or shrub that can grow up to 3-15m tall depending on environmental conditions [1]. The diameter of the stem at breast height can be up to 25 cm [3, 6]. The seed hemisphere is up to ten millimeter in length and seven millimeter in thickness with brownish and wooly floss [5]. The plant has distinctively rounded leaves (8x12cm on long stalk) which are always simple alternate and round with 3-5 lobes [4, 6]. The flowers are 6cm long and yellow and purple in colour with dark purple and dark red centre. The fruit has a round woody capsule of about 3-5cm in diameter. It is clearly divided into 5 segments; each segment has a hemisphere of about 10x7mm in size [4].

Az-garck is very useful in medicine and diet [7]. The most valuable part of the plant is still its edible fruit. They are consumed when they are ripe, dry, or slightly green [2]. The pulp has a sweet, sticky flavour when chewed like chewing gum [8]. They can be boiled and turned into porridge, or they can be soaked in a little water to make jelly. As a vegetable, the leaves are also consumed [4].

It has been claimed that the bark, fruits, leaves, roots, and stems of Az-garck have medicinal qualities and are used to treat and manage a number of diseases [9-10].

The fruit, leaf, stem and root decoctions are sipped with the hope that it can improve infertility and liver related conditions [6]. The ripe fruits are taken as aphrodisiac and also for anemia [11]. The roots are said to induce labour in pregnant women, treat cough and chest pains, as anti-emetic, remedy for mental illness and retained placenta [12-13].

The flesh and seed of Az-garck are rich sources of phytochemicals, including ascorbic acid, carotenoids, tannins, saponins, alkaloids, phenols, etc; according to the submission of Ochokwu and colleagues [4-5].

Bekinbo *et al.* [14] stated that prior to an in depth exploration of therapeutic potentials of herbal plants and products, the focus should be on properly screening, identifying and possibly characterizing the active ingredients of such plants and herbs. Considering this line of thought, the present study set out to evaluate the actual phytochemical/active ingredients composition of the hydroethanol extract of the fruit pulp of Az-garck as well as its toxicity profile.

Materials and Methods

The plant material

The az-garck fruits were acquired from Tula in the Kaltungo Local Government Area of Gombe State and were identified and verified by Dr. Ekeke, Chemezie, a plant taxonomist at the University of Port Harcourt, Nigeria. Samples for herbarium voucher were submitted and voucher number UPH/P/414, was obtained.

Extraction and preparation of plant extract

The fleshy portion of the fruit (pulp) were separated from the seeds and air dried at room temperature (37 ºC), Afterwards, it was pulverized into powdered form using a grinding mill machine. The powdered fruit pulp was soaked in ethanol and water (ethanol: water, 70:30 v/v) and stirred intermittently to form a uniform mixture. The mixture was filtered and concentrated using a rotary evaporator at 60 ºC. The extract was refrigerated at temperature 4 ºC prior to use.

Qualitative Phytochemical Screening of the Extract

The qualitative phytochemical evaluation was done to identify the components of the herbal extract using standard test methods [15]. This includes; tannins, steroids/triterpenes, flavonoids, carbohydrates, alkaloids glycosides.

Fig 1: The fruits of Az-garck and its processing.

Method of Gas Chromatography and Mass Spectrometry (GC-MS) Analysis of the Extract

The GCMS analysis of the hyroethanolic extract of Az-garck fruit pulp was carried out using GC Clarius 500 Perkin Elmer analyzer. Helium (99.99%) was employed as the carrier gas in an Elite-1 (one hundred percent Dimethyl Polysilloxane) column (30 0.25 mm 1 µmdf) equipped with a split mode (10:1) flow rate of 1 ml/min.

With the injector temperature set at two hundred and fifty degree Celsius, a 2 µl injection volume of the sample's ethanol solution was injected into the column. After two minutes, the temperature in the GC oven increased to two hundred degrees Celsius at a rate of ten degrees Celsius per minute without becoming isothermic. For nine minutes, the isothermic condition was maintained at two hundred and eighty degree Celsius with a programme rate of five degree Celsius per minute. The injector and detector temperatures were set at two hundred and fifty degree Celsius and two hundred and eighty degree Celsius respectively. Ion source temperature was maintained at two hundred degree Celsius. At electron ionization of 70eV, the mass spectra were taken. A scan interval of 0.5seconds and fragments from 45-450 amu (Atomic mass unit) was maintained. The total running time was 36 minutes.

Method of the Acute Toxicity (LD_{50}) Investigation

The acute toxicity screening of the Az-garck fruit pulp was determined using the Lorke Method (Lorke, 1983) [16]. Thirty

(30) female Wistar rats were procured and selected into six (6) different groups of five (5) rats each. Graded doses of the extract, including 250 mg/kg bw, 500 mg/kg bw, 1000 mg/kg bw, 1500 mg/kg bw, 2000 mg/kg bw and 3000 mg/kg bw were administered to groups 1 to 6 respectively. The rats were then observed for any possible death or adverse changes in behaviour and other physiological activities for 24 hours. The geometric mean of the lowest toxic dose and highest tolerated dose was used to determine the oral median lethal dose (LD_{50}).

Results

Qualitative phytochemical Screening

The phytochemical screening of hydroethanolic extract of Az-garck fruit pulp showed that secondary metabolites such as Alkaloids, carbohydrates, steroids/triterpene where present while saponins, flavonoids, cardiac glycoside, and phenols were absent. This is indicated in the Table 1 below.

Table 1: Qualitative Phytochemical Constituents of Hydroethanolic Extract of Az-garck Fruit pulp.

Constituents	Inference
Alkaloids	+
Triterpenoid/Steroid	+
Cardiac glycosides	-
Carbohydrates	+
Anthraquinones	-
Saponins	-
Flavonoids	-
Phenolic Constituents	-
Cyanogenic glycoside	-
Fixed oil	-
Phobatannins	-

Table 2: Chemical compounds identified in the Hydroethanolic extract of *Azanza garckeana* fruit pulp.

Name of Compound	Retention Time (RT) (minutes)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
Bicyclo [3.1.1] heptane, 2, 6, 6-trimethyl	13.724	$C_{10}H_{18}$	138.2499	2.70
Hexadecanoic acid, ethyl ester	15.265	$C_{18}H_{36}O$	284.4772	15.75
9, 12-Octadecadienoic acid, ethyl ester	16.821	$C_{20}H_{36}O$	308.4986	3.08
9, 12-Tetradecadien-1-ol, acetate	16.884	$C_{16}H_{28}O_2$	252.39	12.34
Octadecanoic acid, ethyl ester (Methyl Stearate)	17.096	$C_{19}H_{38}O_2$	312.5304	4.50

Table 3: Biological activities of Chemical compounds identified in the Hydroethanolic extract of Az-garck *fruit pulp*.

Name of Compound	Potential biological Effect
Bicyclo [3.1.1] heptane, 2,6,6-trimethyl	A Family of terpenes which are known to possess anticancer antidiabetic, antiviral, anti-depressant, antifungal properties (Cox-Georgian *et al*.2019; Ayu *et al.* 2022) [23]
Hexadecanoic acid, ethyl ester (Palmitic acid)	Hemolytic, 5-Alpha reductase inhibitor, antioxidant, hypocholesterolemic nematicide, chemical, and anti-androgenic flavour (Vedhanayaki and Ramkumar, 2019; Siswadi and Saragih 2021) [27, 28]
9,12-Octadecadienoic acid, ethyl ester (Ricinoleic acid)	Anti-inflammatory, anti-oxidant, anti-microbial, antiviral, wound healing, pain relieving, anti-cancer (Patel *et al*.2016, Ukwubile *et al.*, 2019)
9,12-Tetradecadien-1-ol, acetate	Anti-inflammatory, antioxidant, anti-cancer, anti-diabetic, cardioprotective, neuroprotective
Octadecanoic acid, ethyl ester (methyl stearate)	Antitumor, Anticancer (Reza *et al.* 2021; Ukwubile *et al*. 2019) [29]

****Source**: -Dr. Duke's Phytochemical and Ethnobotanical Databases

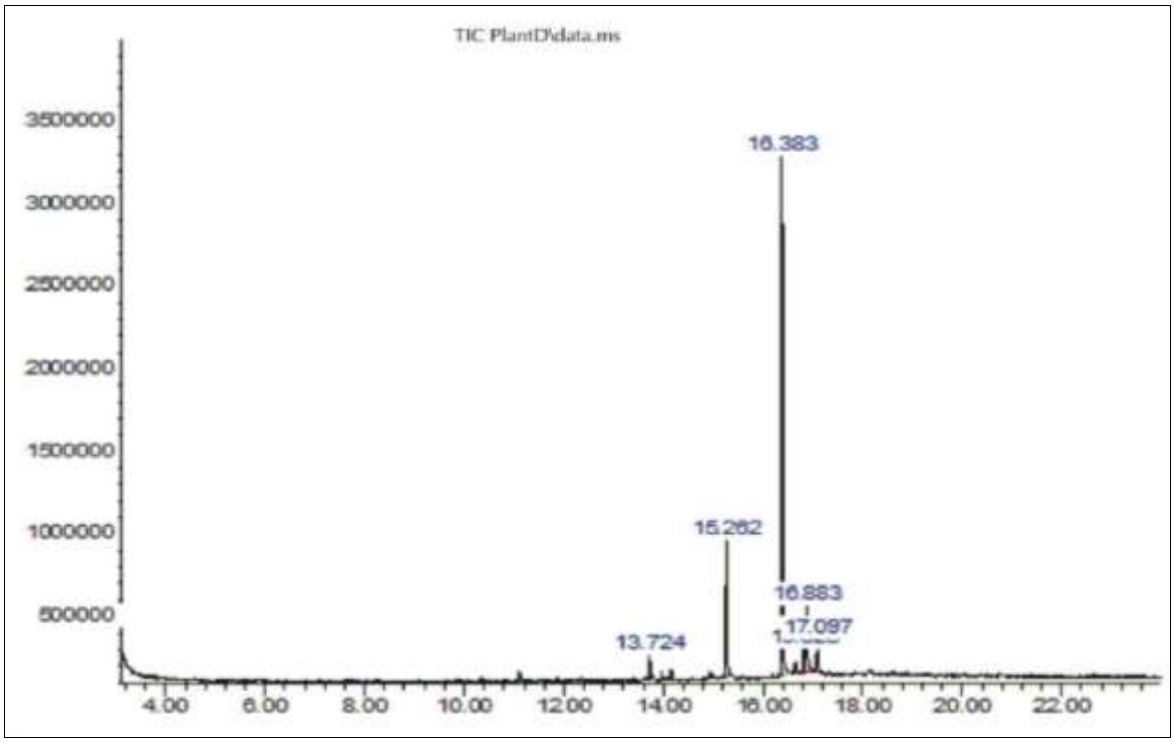

Fig 1: Chromatogram obtained from GC-MS screening of the hydroethanolic extract of Az-garck fruit pulp.

Result on Acute Toxicity Screening

The experiments were done in two phases as shown in Table 2 below:

Table 2: Median lethal dose of hydro-ethanol fruit extract of Az-garck administered orally.

Experiment	Dose (mg/kg)	Number of rats per group	Number of Dead rats after 24 Hours
Phase I	250	5	0
	500	5	0
	1000	5	0
Phase II	1500	5	0
	2000	5	0
	3000	5	1

Discussions

Medicinal plants and herbs are often rich in various phytochemicals which are in turn capable of ameliorating various diseases and illnesses [17-18]. It is important that medicinal herbs should be screened, their active compounds identified, and if possible, be characterized prior to being widely used and commercialized. This will assist in figuring out the possible toxicity, interactions with other therapeutic drugs, and biological processes of action of the herbs [14]. Therefore, the current study evaluated the phytochemical/active component composition of the hydroethanol extract of the Az-garck fruit pulp and its toxicity profile. It produced some significant findings, which are covered in the paragraphs that follow.

The current study's outcome on the qualitative phytochemical screening of the hydroethanolic extract of Az-garck fruit pulp revealed the presence of alkaloids, carbohydrates, and steroids/triterpene. These identified phytochemicals of the extract have been continuously linked to many beneficial/therapeutic and other times, especially in excess, toxic effects in the biological systems [19]. The herb, Az-garck has been shown to have antimicrobial, anti-inflammatory, anti-bacterial effects, anti-viral effects. These qualities could be due to the presence of alkaloids [20]. Alkaloids are also said to be cardioprotective, anti-proliferative and anti-metastic effects on various cancerous cells [21]. On the other hand, steroids/triterpenes present in the plant extract may also be accountable for its antimicrobial and autoimmune benefits [22]. They have strong anti-oxidant and wound healing properties. Triterpenes are also thought to have anti-diabetic properties; numerous studies have demonstrated their ability to inhibit glucose metabolism-related enzymes, stop the onset of insulin resistance, and restore the usual levels of insulin and plasma glucose [22-24]. It is also well known that steroids enhance sexual activity [25]. Based on the GCMS study of Az-garck, approximately five (5) chemicals are present in the extract. The names of these five compounds, their retention time, molecular formula, molecular weight and peak area were also accounted for on Table 2. Several bioactivities bioactivities of the identified active compounds were also presented on Table 3. Hexadecanoic acid, which is the predominant compound amongst other compounds reported have been found to have anti-tumor properties [26]. Additionally, it has been noted to have antimicrobial, hypocholesterolemic, antioxidant, and anti-inflammatory properties. [27-29]. The second most abundant 9, 12-Tetradecadien-1-ol, acetate has also been noted to possess anti-inflammatory, antioxidant, anti-cancer, anti-diabetic, cardioprotective, neuroprotective potentials. These active compounds identified and presented on Table 2, may account for numerous health benefits of this plant and thus their wide ethnobotanic applications in our locale.

According to the results of the acute toxicity test, the rats were only initially restless and occasionally gathered in the cage corners for roughly half an hour after the appropriate doses were administered. After that, the rats were stable. In group 6, which received 3000 mg/kg of the extract, there was just one recorded fatality, and it happened 22 minutes after treatment. The geometric mean of the lowest toxic dose and highest tolerated dose was used to determine the oral median lethal dose (LD_{50}).

$LD_{50} = \sqrt{2000 \times 3000}$

LD_{50} =2449.5mg/kg of Az-garck fruit pulp

Considering the above outcome, the LD_{50} of the extract can be said to be greater than 2000mg/kg bw Az-garck fruit pulp. Thus the effective doses of the hydroethanolic extract of Az-garck fruit pulp, may be <2000mg/kg bw.

Conclusion

The analysis of Az-garck's hydroethanolic fruit pulp extract revealed that the plant contains a wealth of active chemicals that may have a variety of positive medicinal and health benefits. These components could account for a significant portion of the therapeutic results that have been widely praised by conventional medicine practitioners as well as other early scientific studies. This explains why they are often used in our area for ethnobotanical purposes. Even if the identified active ingredients in the hydroethanolic leaf extract of Az-garck may be used for other advantageous purposes, more research should take into account thorough evaluations of their toxicity profile.

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