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ANTI-MICROBIAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF AQUEOUS FRUIT PULP EXTRACTS OF *AZANZA GARCKEANA* (F.HOFFM.)

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ABSTRACT

A study was conducted to investigate the antimicrobial activities and phytochemical components of aqueous fruit pulp extracts of *Azanza garckeana* (F. Hoffm). The fruit of *Azanza garckeana* was obtained from Tula, Kaltungo Local Government Area of Gombe State from which crude fruit pulp extract was prepared. The treatment for the antimicrobial studies consisted of three different concentrations of the crude extract of *A. garckeana* (1000 mg/ml, 500mg/ml and 200mg/ml) while Ketoconazole served as the positive control. All treatments were replicated three times. *Aspergillus niger*, *Rhizopus stolonifer*, *Escherichia coli* and *Staphylococcus aureus* were used to test the antimicrobial activity of the extract. Fruit pulp extract of *A. garckeana* inhibited the growth of both bacteria with the 1000 mg/ml treatment having the highest zone of inhibition of 8.00 mm in *E. coli* and 9.00 mm in *S. aureus.* However, fungi were resistant in all the treatments with no zone of inhibition recorded. From the statistical analysis, there was a significant difference at $p < 0.01$ in the zone of inhibition between the treatments and the control in both bacteria. Quantitative phytochemistry also revealed that saponins, tannins, flavonoids, alkaloids, glycosides and phenols were all present in the crude fruit extract. It is recommended that *A. garckeana* crude fruit pulp extracts can be used as an alternate to synthetic antibacterial drugs. Further research should be carried out to investigate the toxic effect of the fruit as well as to monitor its effective dosages using laboratory animals as well as human clinical trials.

Keywords: *Azanza garckeana;* phytochemistry; fungi; bacteria; antimicrobial activity
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INTRODUCTION

Azanza garckeana (F. Hoffm.) belongs to the Family Malvaceae, which is the distinctive family to which the hibiscus belongs (Michael *et al*., 2015). The plant is locally called '*Goron Tula'* (Hausa, Nigeria). It is also called snotappel (Afrikaans); Chinga, Mukole (Bemba); Azanza, tree hibiscus, snot apple, quarters, wild hibiscus, African chewing gum (English); Muneko (Lozi); Mukole (Lunda); Uxhakuxhaku (Ndebele); Mkole (Nyanja); Mutohwe (Shona); Mtobo (Swahili); Muneko (Tongan) and Morajwa (Tswana) (Mojeremane and Tshwenyane, 2004; Orwa *et al.*, 2009; Ochokwu *et al*., 2014). Most of the Malvaceae in Nigeria are shrubs. *Azanza garckeana* is a valuable edible indigenous fruit tree species found in Gombe State, Nigeria. It is a deciduous shrub or small spreading tree, 3-13 m high, with a diameter at breast height of up to 25 cm (Orwa *et al*., 2009). Michael *et al*. (2015) reported that it grows naturally in semi-arid areas receiving the lowest annual rainfall of 250 mm and the highest rainfall of 1270 mm. Orwa *et al*. (2009) stated that the mean annual rainfall is

between 250 mm to 500 mm at an altitude of 0-1900 m. The fruit has rough and hairy bark; it is grayish-brown, fibrous with longitudinal fissures and brown to yellow slash, young branchlets stellate, tomentose becoming glabrescent when mature. According to Orwa *et al*. (2009), the fruit is 2.5-4 cm in diameter, clearly divided into 5 segments. The fleshy gummy pulp which is generally eaten is a good source of proteins, minerals, fiber, vitamins, and contains five seeds inside with a seed in each segment. The young leaves are bronze in colour and velvety (Mojeremene and Tshwenyane, 2004). It is also found in Sudan, Kenya, Malawi, Mozambique, Namibia, Tanzania, Zimbabwe and Zambia. *A. garckeana* grows in a variety of soils and is found near termite mounds and deserted areas. In Nigeria, it grows in open woodland in the northeastern part of the country (Ochokwu *et al*., 2015).

Mutindi (2014) evaluated antimicrobial activities of crude root extract of *A. garckeana* and pure compounds were isolated from the roots of the species which include gossypol 1, 6, 6-Dimethoxygossypol, 2, 6-Methoxygossypol 3, stigmasterol4, *E*-docosyl 3-(3,4-Dihydroxyphenyl) acrylate and betulinic acid. These were tested against *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus aureus* using ciprofloxacin as the control (Maroyi and Youssef, 2017). Multiple classes of compounds including alkaloids, amino acids, ascorbic acid, carotenoids, cyanogenic glycosides, flavonoids, lipids, phenols, saponins and tannins have been isolated from *A. garckeana* fruits, leaves, roots, seeds and stem bark (Mutindi, 2014; Essiett *et al*., 2011). Some of the documented phytochemicals are recommended by nutritionists because of their health benefits as they are considered to be responsible for positive health benefits. Zhang *et al*. (2015) noted that phytochemicals such as alkaloids, flavonoids, saponins, steroids, tannins and triterpenoids isolated from fruits, vegetables and grains exerted a protective effect against the development of chronic diseases such as cardiovascular diseases (CVD), diabetes and cancers. According to Zhang *et al.* (2015), the protective role of phytochemicals may be associated with their antioxidant activity since overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in the human body is involved in the pathogenesis of many chronic diseases. Essiett *et al*. (2011) isolated amino acids from fruits and leaves of *A. garckeana* with aspartic acid, glutamic acid, leucine and lysine being the most abundant amino acids constituting 9.67-12.97 g/100 g. This study was aimed at determining the antimicrobial activity, the minimum inhibitory concentration and the phytochemistry of the aqueous fruit pulp extract of *Azanza garckeana*.

MATERIALS AND METHODS

Study Area

Antimicrobial activities and determination of minimum inhibitory concentration were carried out in the Department of Botany laboratory, Adamawa State University, Mubi while phytochemical screening was done in the Department of Crop Science laboratory, Adamawa State University, Mubi from September 2019 to January, 2020. Mubi is located between latitude 10º12´N and longitude 13º10´E. It is characterised by two seasons: the wet season which begins in April and ends in October and the dry season which begins in November and ends in March (Adebayo, 2004). The climate is tropical with an average temperature of about 15-42ºC in the dry season, relative humidity of about 10-45% and an annual rainfall of about 1056 mm (Adebayo and Tukur, 1999).

Sample Collection

The fruits of *Azanza garckeana* were obtained from Tula in Kaltungo Local Government Area, Gombe State. The plant was authenticated by a botanist in the Department of Botany, Adamawa State University, Mubi and was kept in the laboratory of the Department until when required. The bacterial test organisms were obtained from Mubi General Hospital while the fungal pathogens were obtained from the laboratory of the Department of Botany, Adamawa State University after isolation from rotten fruits and were identified using morphological and microscopic characteristics.

Preparation of Aqueous Fruit Pulp Extracts

The extraction process was done using the Soxhlet method of extraction described by Habbal *et al*. (2011). Goron Tula was collected from Tula in Kaltungo Local Government Area of Gombe State and brought to Botany Laboratory for drying. The fruits were washed under tap water, rinsed in three changes of sterile distilled water and dried using sterile blotting paper as described by Zakawa *et al*. (2019). They were then allowed to dry under the shade and thereafter ground into powder using mortar and pestle. Three concentrations were prepared: 1000 mg/ml, 500 mg/ml and 200 mg/ml using the formula:

$C_1V_1 = C_2V_2$

Where,

C_1 = Weight used for stock preparation

V_1 = Volume of stock solution required for each concentration

C_2 = Concentration required

V_2 = Volume of distilled water required for each concentration

Antifungal Effect of the Crude Fruit Pulp Extract of *A. garckeana in vitro*

In vitro control of fungal pathogens (*Aspergillus niger* and *Rhizopus stolonifer*) isolated from rotten fruits using aqueous fruit extracts of *A. garckeana* was carried out following the method described by Cheesbrough (2006). Agar well diffusion technique as described by Cheesbrough (2006) and Tizhe *et al.* (2019) was used to determine the antifungal activity of the extracts. The test organisms in overnight incubated nutrient broth were diluted to 0.5% McFarland equivalent standard by serial dilution using sterile distilled water (Cheesbrough, 2006). An 18 ml of Sabouraud Dextrose Agar (SDA) plates (for *Aspergillus niger* and *Rhizopus stolonifer*) that have been checked for sterility were then seeded with 1 ml of the standardised inoculum of each of the fungal isolates in sterile Petri dishes. The crude extracts were tested on the isolates at the concentration of 1000 mg/ml, 500 mg/ml and 200 mg/ml. The seeded plates were allowed to set after a uniform distribution of the inoculum following swirling of the Petri dish. A standard sterile cork borer of 6 mm diameter was used to cut four uniform wells on the surface of the agar. Four wells on each isolate plate were filled with each of the three concentrations of the crude extracts with the aid of a sterile syringe. Ketoconazole was used as control. Each of the treatments was replicated three times and was laid out in a Completely Randomised Design (CRD). The plates were incubated at 37°C for 18-24 h and were observed for zones of inhibition. A zone of clearance around each well signified inhibition and the diameter of each zone was measured in millimetres (mm) with a transparent ruler (Cheesbrough, 2006; Tizhe *et al.*, 2019).

Antibacterial Effect of Crude Fruit Extract of *A. garckeana in vitro*

Agar well diffusion technique as described by Cheesbrough (2006) was used to determine the antibacterial activity of the extracts. The test bacteria (*E. coli* and *S. aureus*) in overnight incubated nutrient broth were diluted to 0.5% McFarland equivalent standard by serial dilution using sterile distilled water (Cheesbrough, 2006). An 18 ml of Nutrient Agar (NA) plate that has been checked for sterility was then seeded with 1ml of the standardised inoculum of each of the bacteria in sterile Petri dishes. The crude extracts were tested on the isolates. The seeded plates were allowed to set after a uniform distribution of the inoculum following swirling of the Petri dish. A standard sterile cork borer of 6 mm diameter was used to cut four uniform wells on the surface of the agar.

Four wells on each isolate plate were filled with each of the treatment concentrations (1000 mg/ml, 500 mg/ml, 200 mg/ml and the control) with the aid of a sterile syringe. Ciprofloxacin was used as control and the treatments were replicated three times in a completely randomised design (CRD). The plates were incubated at 37°C for 18-24 h and observed for zones of inhibition. A zone of clearance around each well signified inhibition and the diameter of each zone was measured in millimetres (mm) with a transparent ruler.

Determination of Minimum Inhibitory Concentration (MIC)

A three-fold double dilution of each of the crude extract concentrations was made based on the results from the antimicrobial sensitivity assays with final concentrations of 1000, 500 and 200 mg/ml, respectively. Sterile tubes containing 5 ml of sterile nutrient broth were inoculated with 0.2 ml each of the test organisms (*E. coli* and *S. aureus*) after adding 0.5 ml of each of the extract concentrations, thus making three tubes of each of the extract concentrations for an organism. Also, the sterility of the broth was tested as well as the viability of the organisms. Equivalent volumes of the broth to which was added the organisms but no extracts were used to test for the viability of the organisms while a broth tube free of the test organisms and the extract was used to test the sterility of the broths (Mohan and Pandey, 2016).

Quantitative Phytochemical Analysis

The quantitative analysis of phytochemicals present in the aqueous fruit pulp extract of *Azanza garckeana* was carried out for alkaloids, tannins, phenols, flavonoids, saponins and glycoside using the methods described by Harbone and Baxter (1993), Okwu (2001), Rahilla *et al.* (1994), Sofowora (1982) and Ahmad and Beg (2001) as reported by Al-Daihan *et al*. (2013).

Experimental Design /Data analysis

The treatments consisted of three concentrations of Aqueous Fruit Pulp extracts of *A. garckeana* (*E. coli* and *S. aureus*) and Ketoconazole as control which were replicated three times and laid out in a Completely Randomised Design (CRD). Data obtained from the study were subjected to one-way Analysis of Variance (ANOVA) using MINITAB Computer software programme. Means were separated using Duncan's Multiple Range Test (DMRT) at $p < 0.01$.

RESULTS

Quantitative Phytochemical Screening of Aqueous Fruit Pulp Extracts of *A. garckeana*

The result of quantitative phytochemical screening of aqueous fruit extract of *Azanza garckeana* is presented in Table 1. The result indicated that the aqueous fruits pulp extract of *Azanza garckeana* contained saponins (2.46%), tannins (3.85%), flavonoids (1.76%), alkaloids (6.11%), glycosides (0.77%) and phenols (16.54%).

Table 1: Result of quantitative phytochemical screening of aqueous fruits extract of *A. garckeana*

Phytochemicals	Composition (%)
Saponins	2.46
Tannins	3.85
Flavonoids	1.76
Alkaloids	6.11
Glycosides	0.77
Phenols	16.54

The Antifungal Activities of Aqueous Fruit Pulp Extract *A. garckeana*

The antifungal activities of *A. garckeana* showed a significant difference between the control and other treatments. However, the result showed that *A. garckeana* fruit pulp extract had no antifungal effect on the organisms tested (*A. niger* and *R. stolonifer*) as shown in Table 3.

Table 3: Antifungal activities of aqueous fruit pulp extract of *A. garckeana*

	Zone of inhibition (mm)	
Treatment (mg/ml)	*A. niger*	*R. stolonifer*
1000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
500	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
200	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control (+)	11.33 ± 0.66^{b}	11.00 ± 0.58^{b}

Means followed by the same superscript in the same column are not significantly different from each other (DMRT) at $p<0.01$

The Antibacterial Activities of Aqueous Fruit Pulp Extract of *A. garckeana*

The Analysis of Variance (ANOVA) on the antibacterial activities of aqueous fruit pulp extract of *A. garckeana* showed that there was a significant difference between the control and all other treatments for both organisms (*E. coli* and *S. Aureus*) at a probability level of $p<0.01$. The control resulted in the highest growth inhibition for both *E. coli* and *S. aureus* (11.00) while there was no inhibition at 200 mg/ml (0.00). The treatment with 1000 mg/ml resulted in 8.00 growth inhibition in *E .coli* and 9.00 in *S. aureus* while it was 6.00 in both organisms at 500 mg/ml as shown in Table 2.

Table 3: Antibacterial activities of aqueous fruit pulp extract of *A. garckeana*

Treatments (mg/ml)	*E.coli*	*S. aureus*
1000	8.00 ± 0.58^{a}	9.00 ± 0.58^{a}
500	6.00 ± 0.58^{a}	6.00 ± 0.58^{a}
200	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Control(+)	11.00 ± 0.58^{c}	11.33 ± 1.20^{c}

Means followed by the same superscript in the same column are not significantly different from each other (DMRT) at $p<0.01$

Minimum Inhibitory Concentration (MIC) of Aqueous Fruit Extract on Test Organisms

The result of the minimum inhibitory concentration of aqueous extract on test organisms is presented in Table 4. The result showed that 1000 mg/ml resulted in maximum inhibitory concentration with no growth of *E. coli* and *S. aureus*, followed by 500 mg/ml with light growth, while 200 mg/ml showed heavy turbidity of growth. The minimum inhibitory concentration for *A. niger* and *R. stolonifer* showed that none of the concentrations of the extract had inhibitory activity on the test fungi.

Table 4: Minimum inhibitory concentration of aqueous fruit pulp extract against test organisms

Treatment (mg/ml)	*E.coli*	*S.aureus*	*A. niger*	*R.stolonifer*
1000	-	-	R	R
500	+	+	R	R
200	+++	+++	R	R

KEY: - = No turbidity (no growth), + = Turbidity (light growth), +++ = Heavy turbidity,
R= Resistant to extract.

DISCUSSION

Saponins, tannins, flavonoids, alkaloids, glycosides and phenols were all present in the aqueous fruit pulp extract of *A. garckeana*. This finding agrees with Mutindi (2014) and Essiett *et al*. (2011) who reported multiple classes of compounds including alkaloids, amino acids, ascorbic acid, carotenoids, cyanogenic glycosides, flavonoids, lipids, phenols, saponins and tannins from fruits, leaves, roots, seeds and stem bark of *A. garckeana*. It has been reported that *A. garckeana* is used as medicine by the rural dwellers. Root infusion of *A. garckeana* is taken orally as a remedy for chest pains, cough and menstrual pain in Nigeria, Zimbabwe and Kenya (Essiett *et al*., 2011). The root and stem bark decoction of *A. garckeana* is taken orally as a remedy for gonorrhoea, sexually transmitted diseases and syphilis in Malawi, Nigeria and Zambia (Essiett *et al*., 2011). The leaf, stem, root decoction or ripe fruits of *A. garckeana* are taken orally as a remedy for infertility and liver problems in Botswana, Kenya, Malawi and Nigeria (Mahboubi *et al*., 2015).

The result of this study revealed that 1000 mg/ml and 500 mg/ml aqueous fruit pulp extract of *Azanza garckeana* significantly inhibited the growth of *E. coli* and *S. aureus*. The results showed that the fruit pulp of *A. garckeana* contained bioactive agents that exhibited antimicrobial properties on both Gram-positive and Gram-negative bacteria. The fruit pulp has shown a broad-spectrum activity for its effectiveness in the therapy of some of the acclaimed antimicrobial properties. This finding agrees with Mutindi (2014) who evaluated antimicrobial activities of crude root extract of *A. garckeana* and pure compounds isolated from the roots of the species including gossypol 1, 6, 6-Dimethoxygossypol 2, 6-Methoxygossypol 3, stigmasterol4, *E*-docosyl 3-(3, 4-Dihydroxyphenyl) acrylate and betulinic acid were tested against *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus aureus* using ciprofloxacin as the control (Maroyi and Youssef, 2017). Similarly, Gupta *et al*. (2010) investigated the antibacterial activity of five ethanolic and aqueous plant extracts against *S. aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis;* the results showed that the ethanolic extracts of four plants (*Achyranthes aspera*, *Cynodon dacynodon*, *Lantana camara* and *Tagetes patula*) were effective against all tested microorganisms with MICs ranging from 25 to 125 mg/ml. Sapkota *et al*. (2012) studied the antibacterial effect of guava leaves, garlic and ginger on some human microbial pathogens and concluded that ginger was only effective against *S. aureus* while guava and garlic were effective against all tested microorganisms. Also, Mahboubi *et al*. (2015) reported the antimicrobial activity of ethanolic *Punica granatum* extract and its fractions showed high antibacterial activity on Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli* and *S. typhi*) causing food poisoning; these extracts can be used for prevention of food-borne diseases or as a preservative in the food industry.

CONCLUSION

This study has shown that the aqueous fruit pulp extract of *Azanza garckeana* has antimicrobial properties on *Staphylococcus aureus* and *Escherichia coli*. The study also revealed that fruit pulp extract of *Azanza garckeana* had no antimicrobial effect on fungal organisms (*Aspergillus niger* and *Rhizopus stolonifer*), but acted as a growth medium for the organisms which appeared to grow faster in the extract. The fruit pulp extracts were shown to possess a significant amount of phytochemicals such as saponins, tannins, flavonoids, alkaloids, glycosides and phenols. These findings are instructive in view of the problems of emerging and re-emerging resistant strains of microorganisms. *Azanza garckeana* plant is readily available in Nigeria and could serve as an alternative to curing of microbial infections at a lower cost. It is hereby recommended that further research should be carried out to investigate the toxicity effect of the fruit of *A. garckeana* as well as to monitor its effective doses using laboratory animals as well as human clinical trials. Investigation should also be carried out to determine if *A. garckeana* crude fruit pulp extract could serve as media for fungal growth.

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