Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Pharmacological activities of *Azanza garckeana* (Goron Tula) grown in Nigeria

Abubakar Awwal Yusuf[1], Bashir Lawal[2,3*], Saidu Sani[4], Rahinat Garba[2], Bala Alkali Mohammed[2], David Bini Oshevire[5] and Daniel Anuoluwa Adesina[2]

Abstract

Background: The present study evaluated the phytochemical profiles, anti-oxidants, antimicrobial, anti-inflammatory, analgesic, anti-arthritic and wound healing effect of methanol and ethyl-acetate extracts of *Azanza garckeana*.

Results: Methanol extract had higher phenol, flavonoids and alkaloids concentrations, ferric reducing antioxidant power (FRAP) and hydroxyl radical scavenging activities than ethyl-acetate extract. Ethyl-acetate and methanol extracts had IC_{50} of 119.40 μg/mL and 133.49 μg/mL respectively against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radicals. The extracts were more active against *Klebsiella pneumonia* while the least activity was recoded against *Bacillus subtilis* with methanol been most potent than ethyl-acetate. The IC_{50} value of methanol extract in inhibition of protein denaturation were 310.44 μg/mL and 315.27 μg/mL while IC_{50} value of 354.74 μg/mL and 349.57 μg/mL were recorded for membrane stabilization effect in bovine serum albumin (BSA) and egg albumin (EGA) assays respectively. There was dose dependent in vivo anti-inflammatory and analgesic activities with highest percentage paw oedema inhibitions of 51.68% and analgesia of 46.36% at 600 mg/kg bw of the methanol extract. The extract caused dosed independent increase percentage wound closure with percentage wound contraction range between 65.24 ± 2.46 and 69.68 ± .2.54 compare to untreated wound (15.35 ± 0.35 to 50.35 ± 2.35%).

Conclusion: This study lends pharmacological support to folkloric usage of *Azanza garckeana* in the treatment and management of several human disease.

Keywords: *Azanza garckeana*, Phytochemicals, Pharmacological activities

Introduction

Medicinal plants play a vital function in both development and production of new drugs [1]. This is also in line with the report of World Health Organization (WHO), which indicated that about 80% of global's population in developing countries depends on natural products for basic and primary healthcare need [2]. Furthermore, more than 20% of conventional and standardize drugs have a phytochemical backbone [1]. Virtually all human diseases including infectious disease, inflammatory, analgesic, diabetics, malaria, hepatorenal diseases and disease associated with oxidative stress have been treated or manage with medicinal plants [3–7]. Furthermore, the safety of medicinal plants as oppose to the undesirable side effect associated with the use of synthetic drug made medicinal plants a worthy alternative to synthetic drugs [8]. The pharmacological activities of medicinal plants have been attributed to the presence of secondary metabolites including saponins, phenols, flavonoids, tannins, terpenoids and many more [9].

The emergence and dissemination of multidrug resistant (MDR) strain of pathogenic organism have become a major threat of public health concern and thus only few effective antimicrobial agents are available for treatments of infection diseases [10]. The use of anti-microbial

* Correspondence: lawal.b@st.futminna.edu.ng
[2]Department of Biochemistry, Federal University of Technology Minna, Minna, Nigeria
[3]PhD Program for Cancer Molecular Biology and Drug Discovery, Taipei Medical University and Academia Sinica, Taipei 111, Taiwan
Full list of author information is available at the end of the article

agents from natural product would be useful in reducing the global burden of infectious diseases [11].

Accumulation of free radicals e.g. hydroxyl radicals, hydrogen peroxide and superoxide anions occur during excessive oxygen supply or its insufficient reduction during aerobic metabolism [12]. The accumulation of these free radicals leads to oxidative stress which in turn cause oxidative damage to biomolecules and consequently plays etiologic role in several metabolic and degenerative diseases [13]. Phytochemicals have demonstrated a protective role against the free radicals and reduce the risk of oxidative stress induced diseases.

The *Azanza garckeana* (F. Hoffm.) Exell and Hillc commonly known as Goron Tula, (kola of Tula) in Hausa, belong to the family Malvaceae. In Nigeria, it is grown only in Tula village of Gombe State. It is a multipurpose edible fruit of tropical Africa. It is an important medicinal and food plant commonly used in Northern Nigeria as herbal medicines [14]. *Azanza garckeana* has also been reportedly use in traditional medicine for treatments of management of more than 20 human diseases and ailments. The plant is used as herbal remedy for diseases like cough, chest pains, infertility, menstruation abnormalities, sexually transmitted infections and hepatic impairments [15, 16]. Multiple classes of bioactive metabolites including amino acids, alkaloids, ascorbic acid, carotenoids, flavonoids, glucosides, phenols, lipids, tannins and saponins have been isolated from *A. garckeana* [17]. In the present study, we evaluated the phytochemical profiles and pharmacological properties of methanol and ethyl-acetate extracts of *Azanza garckeana.*

Material and method

Plant collection

The *Azanza garckeana* were collected from Gombe village, in Gombe State, Nigeria in the month of July, 2019. The plant was identified by a Botanist in the Department of Biological Science, Ibrahim Badamasi Babangida University, Lapai. Niger State.

Experimental animals

Healthy albino mice and rats were procured from Animal Breeding unit of Ahmadu Bello University Zaria, Nigeria. The mice were used for analgesic study while the rats were used for anti-inflammatory and wound healing studies. The animals were maintained under standard laboratory conditions with access to commercial feed pellets (growers) and water ad libitum. Animals handling and experimentations were in compliance with internationally standard (NIH Publication No. 85–23, 1985). The animals were fasted 12 h before the commencement of any study.

Chemicals and reagents

Ascorbic acid (Merck Co.), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were product of Sigma-Aldrich Co. All other chemicals were of analytical grade.

Sample preparation and extraction

The *Azanza garckeana* fruit sample was air-dried at room temperature (37 °C) and finally grounded using a grinder mill. Extraction of the plant materials were carried out with methanol and ethyl-acetate using Soxhlet apparatus and the resulting extracts were concentrated using rotary evaporator.

Screening for secondary metabolites

The methanol and ethylacetate extracts of *Azanza garckeana* were analyzed quantitatively for the presence of some secondary metabolite including alkaloids, tannins, saponins, phenols, and flavonoids using standard procedures [18–20].

Assay for antibacterial activity

Anti-microbial effect of the methanol and ethyl-acetate extracts of *Azanza garckeana* at varying concentrations (25–100 μg/mL) against *Klebsiella pneumoniae, Pseudomonas aeruginisa, Bacillus subtilis* and *Escherichia coli*. Antibacterial activity of the extract was carried out using agar-well diffusion method as described by Tsado et al., [21]. A broth microdilution method [22], was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract in triplicates.

Antioxidants activities

Methanol and ethyl-acetate extracts of *Azanza garckeana* at varying concentrations (12.5–100 μg/mL) were evaluated for antioxidants activities using 2, 2′-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay [23], Fe3+ ion reducing power assay and $OH^{\bullet}$ scavenging assay according to the method described by Halliwell and Gutteridge, [24] and Oyaizu [25]. The extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition (%) against extract concentration. Ascorbic acid at the same concentrations was used as the reference antioxidants.

Evaluation of in vitro anti-arthritic activity

Antiarthritic activity of the methanol and ethyl-acetate extracts were evaluated by serum bovine albumin and egg albumin methods. In each of the models, inhibition of protein denaturation and effect on membrane stabilization were evaluated at different extracts concentrations (100 – 500 μg/ ml). The protocol described by Shilpa et al. [26] was the adapted for inhibition of protein denaturation assay while the method described by

Anosike et al., [27] were used for membrane stabilization model. The percentage activity of the extracts in each model were calculated using the formula below

$$\textbf{Membrane stabilization} = 100 - \frac{(\text{Absorbance of test solution} - \text{Absorbance of control}) X\ 100}{\text{Absorbance of test solution}}$$

$$\textbf{Percentage inhibition} = \frac{(\text{absorbance of control} - \text{Absorbance of sample})\ X100}{\text{Absorbance of control}}$$

Analgesic activity

Analgesic activity of the extracts at different concentrations (150, 300 and 600 mg/kg bw) were assessed in mice using acetic acid (0.75% v/v) models by the method of Koster et al. [28]. Acetyl salicylic acid (150 mg/kg bw) as reference drug. The number of abdominal constrictions induced by acetic acid was counted after 5 min. Observations were made over 10 min and mean value for each group calculated. Percentage inhibition of abdominal constriction by the plant extract and acetyl salicylic acid (ASA) was determined in relation to the control. Acetyl salicylic acid (ASA) equivalent was also calculated.

Anti-inflammatory activity

The anti-inflammatory activity of the extract was tested using egg albumin induced paw oedema in rats [29]. The increase in volume (cm^3) of the hind paw was measured with a LETICA digital Plethysmometer (LE 7500) before and at 30 min interval after the injection of egg albumin for a period of 150 min. Control rats received an equivalent amount of normal saline while acetyl salicylic acid (150 mg/kg bw) served as reference. The percentage inhibition of oedema was calculated for each dose.

In vivo wound healing effect

Following diethyl ethe anaesthetized. A full thickness wound (1.5 × 1.5 cm) was made on a shaved dorsal area. In vivo wound healing effect of the extract were evaluated by topical administration of the extracts at 150, 300 and 600 mg/kg bw in soft paraffin to the rats excised wound according to the method **of** Sasidharan et al. [30]. Tetracycline were used as the standard drug**.** The wound area of each animal was measured on days 3, 6,9 and 15 post-surgery. The decrease in wound diameters during the healing process was measured with an analytical perimeter. The percentage wound healing was calculated using equation: [(A0 − At)/A0] × 100 = % of wound closure. Where A0 is the original wound area, and At is the area of wound over time.

Table 1 Phytochemical composition of methanol and ethylacetate extracts of *Azanza garckeana*

Phytochemicals	Methanol (mg/100 g)	Ethyl-acetate (mg/100 g)
Total phenol	34.32 ± 2.34	25.34 ± 0.32
Total flavonoids	13.45 ± 0.89	7.65 ± 0.24
Tannins	8.65 ± 0.94	15.23 ± 0.67
Alkaloids	43.24 ± 2.95	32.34 ± 3.24
Saponins	5.43 ± 0.67	5.35 ± 0.67

Data are Mean ± SEM of triplicate determination

Table 2 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging activities of Methanol and ethyl acetate extract of *Azanza garckeana*

Concentration (μg/mL)	Methanol extract	Ethylacetate extract	Ascorbic acid
100	46.61 ± 2.17 ^{a}	46.72 ± 0.02 ^{b}	93.45 ± 0.56 ^{c}
50	40.68 ± 0.02 ^{a}	46.60 ± 0.04 ^{b}	90.46 ± 0.46 ^{c}
25	34.64 ± 0.05 ^{a}	39.47 ± 0.10 ^{a}	62.45 ± 0.46 ^{b}
12.5	38.92 ± 0.24 ^{a}	38.32 ± 0.05 ^{a}	37.56 ± 0.21 ^{a}
IC_{50}	**133.49**	**119.40**	**18.48**

Values are mean ± SEM of 3 determinations. Values along the same row with different superscripts are significantly different ($p < 0.05$)

Statistical analysis

The analysis was performed using SPSS statistical package for WINDOWS (version 21.0; SPSS Inc., Chicago). Data were expressed as the Mean ± SEM of three determination. Results were subjected to ANOVA followed by DMRT. Statistically significant was consider at $p < 0.05$.

Results

Phytochemical composition

Phenols is the most abundant phytochemicals in both methanol (34.32 ± 2.34 mg/100 g) and ethylacetate (25.34 ± 0.32 mg/100 g) extracts of *Azanza garckeana* while saponins (5.43 ± 0.67 and 5.35 ± 0.67 mg/100 g) was the least in both samples respectively (Table 1). Methanol had higher amount of phenol, flavonoids and alkaloids while ethylacetate had higher tannins.

Invitro antioxidants activities

The methanol and ethyl-acetate extract of *Azanza garckeana* promoted an increase inhibition of the DPPH radical with increase extract concentrations. Ethylacetate extract had the least IC_{50} of 119.40 μg/mL while the

Table 3 Ferric Reducing Antioxidant Activity of Methanol and ethyl acetate extract of *Azanza garckeana* whole pulp

Concentration (μg/mL)	Methanol extract	Ethylacetate extract	Ascorbic acid
100	0.31 ± 0.02^{a}	0.78 ± 0.00 ^{b}	1.98 ± 0.32 ^{c}
50	0.28 ± 0.02 ^{a}	0.54 ± 0.10 ^{b}	1.78 ± 0.02 ^{c}
25	0.24 ± 0.01 ^{a}	0.32 ± 0.02 ^{b}	1.20 ± 0.23 ^{c}
12.5	0.13 ± 0.04 ^{a}	0.26 ± 0.03 ^{b}	0.75 ± 0.03 ^{c}

Values are mean ± SEM of 3 determinations. Values along the same row with different superscripts are significantly different ($p < 0.05$)

Table 4 Hydroxyl radical scavenging activity of methanol and ethyl acetate extract of *Azanza garckeana* whole pulp

Concentration (μg/mL)	Methanol extract	Ethylacetate extract	Ascorbic acid
100	68.76 ± 2.34 ^{b}	57.68 ± 3.24 ^{a}	79.45 ± 1.98 ^{c}
50	47.68 ± 3.35 ^{b}	28.76 ± 1.23 ^{a}	65.46 ± 2.46 ^{c}
25	25.46 ± 3.45 ^{b}	13.45 ± 2.34 ^{a}	36.56 ± 1.68 ^{c}
12.5	13.35 ± 1.36 ^{b}	9.46 ± 1.23 ^{a}	22.35 ± 1.23 ^{c}

Values are mean ± SEM of 3 determinations. Values along the same row with different superscripts are significantly different ($p < 0.05$)

methanol extract had IC_{50} of 133.49 μg/mL compare to ascorbic acid (18.48 μg/mL) (Table 2). Ferric reducing antioxidant power assay showed that both methanol and ethyl acetate extracts of *Azanza garckeana* had ferric reducing antioxidant power that increases with extract concentration. However, ethyl-acetate extract exhibited higher FRAP activities than methanol extract (Table 3). Similarly, the hydroxyl radical scavenging ability of the methanol extract was found to be higher than that of the ethylacetate extract (Table 4).

Antimicrobial activities of *Azanza garckeana*

The diameter of the zone of inhibition of methanol and ethyl-acetate extracts of *Azanza garckeana* against the organism investigated increases as the extracts concentrations increases from 25 μg/mL to 100 μg/mL. The extract was more active against *Klebsiella pneumonia* (18.89 ± 0.32 mm) while the least activity was recoded against *Bacillus subtilis* (2.12 ± 0.61 mm) at the highest concentrations (100 μg/mL) (Table 5). The MIC of Methanol extract ranged between 12.5 and 75 μg/mL for methanol extract and between 50 and 75 μg/mL for ethyl-acetate. The MBC on the other hand ranged between 12.5 and 75 μg/mL and also between 50 and 100 μg/mL for methanol and ethyl-acetate extract respectively (Table 6).

Invitro anti-arthritic activity

The extract exhibited increase inhibition of protein denaturation and increase membrane stabilization effect with increase extract concentration in both BSA and EGA models. The IC_{50} value recorded for the extract in inhibition of protein denaturation were 310.44 μg/mL and 315.27 μg/mL in BSA and EGA assays respectively. The membrane stabilization effect was recorded with IC_{50} value of 354.74 μg/mL and 349.57 μg/mL in BSA and EGA assays respectively (Table 7).

In vivo anti-inflammatory and analgesic activities

There was dose dependent reduction in the paw oedema in groups treated with the plant extract and standard drug. The percentage inhibition of paw oedema for the crude extract were 43.82%, 47.19% and 51.68% at 150, 300 and 600 mg/kg bw while diclofenac caused 71.91 paw oedema inhibition (Table 8). The percentages analgesia of 25.01%, 30.54 and 46.36% at the dose of 150, 300 and 600 mg/kg body weight respectively was observed while the standard acetyl salicylic acid (ASA) treatment caused percentages of analgesia 68.66% (Table 9).

Wound healing activities

The percentage wound contraction in both treated and untreated rats increases with time. Untreated rats had significantly the lowest percentage wound closure (15.35 ± 0.35 to 50.35 ± 2.35%). Rats treated with the extract had percentage wound contraction range between 65.24 ± 2.46 and 69.68 ± .2.54 while rats treated with standard drug tetracycline had the highest wound closure rate of 31.56 ± 1.24 to 94.24 ± 3.68%. The extract caused dosed independent increase percentage wound closure (Table 10).

Discussion

Medicinal plants are considered rich resource of bioactive metabolites with potential for drug discovery and development. These bioactive metabolites are of different type, but the most common of these metabolites are phenols, alkaloids, flavonoids, glycosides, and terpenes [31]. Generally, the quality and quantity of secondary metabolites strongly influence the pharmacological properties of medicinal plants [32]. The phenolic compounds considerably occur in plant and vary across plant species and parts [33]. The antioxidant potential of medicinal plants has been attributed to the redox effect of phenolic compounds which scavenge single oxygen, donate proton and act as reducing agents [34]. In the current study, the total flavonoids and phenolics detected in methanol and ethyl-acetate extract

Table 5 Zones of inhibition of methanol and ethyl-acetate extracts of *Azanza*

Conc (μg/mL)	*E. coli*		*P. aeruginosa*		*K. pneumonia*		*B. subtilis*	
	Methanol	Ethyl-acetate	Methanol	Ethyl-acetate	Methanol	Ethyl-acetate	Methanol	Ethyl-acetate
25	2.12 ± 0.40^{a}	1.27 ± 0.22 ^{a}	–	–	5.22 ± 0.18^{a}	–	–	–
50	6.44 ± 0.25 ^{b}	5.34 ± 0.28 ^{b}	–	3.14 ± 0.21^{a}	6.44 ± 0.11 ^{b}	2.04 ± 0.03 ^{a}	–	6.19 ± 0.02 ^{a}
75	6.90 ± 0.56 ^{b}	7.21 ± 0.71^{bc}	3.18 ± 0.19^{a}	9.05 ± 0.21 ^{b}	13.90 ± 0.04 ^{c}	5.05 ± 0.41 ^{b}	2.56 ± 0.33^{a}	6.82 ± 0.29 ^{a}
100	7.25 ± 0.17 ^{b}	9.46 ± 0.19 ^{b}	7.23 ± 0.67 ^{b}	15.17 ± 0.19 ^{c}	18.89 ± 0.32 ^{d}	8.08 ± 0.20 ^{c}	2.12 ± 0.61 ^{a}	9.26 ± 0.31 ^{b}
Control	16.44 ± 0.25 ^{c}	16.44 ± 0.25 ^{c}	28.50 ± 0.40 ^{c}	28.50 ± 0.40 ^{d}	28.00 ± 0.00 ^{e}	28.00 ± 0.00 ^{d}	16.78 ± 0.67 ^{b}	16.78 ± 0.67 ^{c}

Values are Mean ± SEM of triplicate determinations. Data followed by different superscript alphabet *a*long the column were significantly different ($p < 0.05$)

Table 6 Minimum inhibitory and minimum bactericidal concentration of methanol and ethyl-acetate extracts of *Azanza garckeana* against some pathogenic organism

Test organism	MIC		MBC	
	Methanol extract	Ethyl-acetate extract	Methanol extract	Ethyl-acetate extract
Pseudomonas aeruginosa	75	50	75	100
Bacillus subtilis	75	50	75	75
Klebsiella pneumoniae	12.5	50	12.5	50
Escherichia coli	25	25	25	50

of *Azanza garckeana* corresponded with the antioxidant, antimicrobial and other biological activity observed.

The effect of free radicals in the pathogenesis of infections and diseases has been documented [35]. The balance homeostasis between the free radicals and antioxidants are vital in the defense mechanisms of the body against these diseases [36]. However, negative shift in favor of the free radicals may result in oxidative stress that catalyzes the proliferation of diseases. Literatures indicated that natural antioxidants can prevent or reverse abnormal health effect associated with antioxidants and oxidative stress [31]. With such a scenario, shifting to natural antioxidants is inevitable. In this study, methanol and ethyl-acetate extract of *Azanza garckeana* exhibited noteworthy DPPH scavenging activities, thus providing scientific validation for their uses in treatments of various human diseases in traditional medicine [15]. Based on the IC_{50} values (Table 2), ethyl-acetate extract was more potent than the methanol extract. This is an indication that the ethyl-acetate extract of *A. garckeana* possess higher ability to donate protons to a free radical and thus could be used as primary antioxidants since the antioxidant effect of extracts on DPPH is speculated to be due to their ability to donate hydrogen to free radicals [35].

Table 7 Effect of ethyl-acetate pulp extract of *Azanza garckeana* on heat induced protein denaturation

		Inhibition of Protein Denaturation		Membrane Stabilization Effect (%)	
	Concentration (μg/mL)	BSA Assay	EGA Assay	BSA Assay	EGA Assay
Extract	100	21.20 ± 0.84	23.87 ± 0.35	30.08 ± 0.65	30.56 ± 0.54
	200	32.90 ± 0.56	36.71 ± 0.86	35.89 ± 0.48	35.21 ± 0.78
	300	50.43 ± 0.37	46.98 ± 0.17	40.98 ± 0.36	41.67 ± 0.26
	400	59.89 ± 0.97	61.98 ± 0.98	52.87 ± 0.49	55.65 ± 0.87
	500	75.25 ± 0.98	73.87 ± 0.45	65.87 ± 0.67	68.97 ± 0.26
		310.44	315.27	354.74	349.57
Diclofenac	100	90.06 ± 0.46	89.05 ± 0.35	83.24 ± 0.79	86.79 ± 0.46

Data are express as Mean ± SEM of three determinations

Table 8 Anti-inflammatory effects of crude methanol extract of *Azanza garckeana* against egg albumin induced paw oedema

Vol. of hind paw(cm^3) at different times (min)							
Treatment	Dosage (mg/kg bw)	30	60	90	120	150	% Inhibition
Azanza garckeana	150	0.58	0.54	0.52	0.50	0.50	43.82
Azanza garckeana	300	0.56	0.50	0.48	0.48	0.47	47.19
Azanza garckeana	600	0.58	0.50	0.48	0.46	0.43	51.68
Diclofenac (150)	150	0.54	0.48	0.40	0.33	0.25	71.91
Normal saline	20	0.77	0.76	0.81	0.85	0.89	–

Similarly, both extracts exhibited FRAP and hydroxyl radical scavenging ability with ethyl-acetate extract exhibiting higher FRAP activities and methanol extract exhibiting higher hydroxyl radical scavenging activities (Tables 3 and 4). Findings from the present study concurred with previous studies [35, 37] which reported that the FRAP activity from different solvent extracts of medicinal plants was concentration-dependent. Mshelia et al. [37] also reported that petroleum ether, methanol, acetone and ethyl acetate stem bark extracts of *A. garckeana* exhibited significant DPPH radical scavenging activity with methanol stem bark exhibiting the most potent effect ($IC_{50} < 100$ μg/mL). The ability of plant extracts to transform Fe^{2+} to Fe^{3+} could be attributed to the presence of hydrophilic polyphenolic compounds in the extracts.

The interest in the use of medicinal plants as oral remedy for bacteria and infection disease have increase in recent decades [38]. Although, the antimicrobial activities of root and stem extracts of *A. garckeana* have been study, the fruit extract have not previously been thoroughly investigated. Results from the present study indicated that methanol and ethyl-acetate extracts of *Azanza garckeana* exhibited significant antimicrobial activity. The extract was more active against *Klebsiella pneumonia* (18.89 ± 0.32 mm) while the least activity was recoded against *Bacillus subtilis* (2.12 ± 0.61) at the highest concentrations (100 μg/mL) (Table 5).

The antibacterial activities of root extract of *A. garckeana* against *Enterococcus faecalis, E. coli*, ***S. aureus*** and *Enterococcus faecium* has been reported with minimum

Table 9 Analgesic effects of crude methanol extract of *Azanza garckeana*

Treatment	Mean Abdominal Constriction	% Inhibition
150 mg/kg bw *Azanza garckeana*	28.45 ± 0.57	25.01
300 mg/kg bw *Azanza garckeana*	26.35 ± 2.35	30.54
500 mg/kg bw *Azanza garckeana*	20.35 ± 1.89	46.36
150 mg/kg bw Acetyl salicylic acid	11.89 ± 0.67	68.66
Normal saline	37.94 ± 1.34	–

Data are Mean ± SEM of triplicate determination

Table 10 Percentage wound contraction in rats post infliction of excision wounds

	Treatment periods (Days)				
	3	6	9	12	15
Negative Control	0	0	15.35 ± 0.35^{a}	34.35 ± 1.345^{a}	50.35 ± 2.35^{a}
150 mg/kg bw *A. garckeana*	13.00 ± 0.24^{a}	31.46 ± 0.34^{a}	46.76 ± 2.35^{b}	56.67 ± 2.45^{b}	69.68 ± .2.54^{b}
300 mg/kg bw *A. garckeana*	28.00 ± 1.23^{c}	42.46 ± 1.24 ^{b}	50.32 ± 2.06^{b}	59.36 ± 1.34^{b}	67.24 ± 4.67^{b}
600 mg/kg bw *A. garckeana*	19.45 ± 0.46^{b}	34.27 ± 2.34^{c}	46.78 ± 4.56^{b}	54.76 ± 2.46^{b}	65.24 ± 2.46^{b}
Tetracycline	31.56 ± 1.24 ^{c}	50.32 ± 1.35^{d}	65.24 ± 3.46^{c}	81.34 ± 4.65^{c}	94.24 ± 3.68^{c}

Data are express as Mean ± SEM of three determinations. Values along the same column followed by different superscript alphabet were significantly different ($p < 0.05$)

bactericidal concentration (MBC) values ranging from 0.89–20 μg/mL [39]. The variation in MIC and MBC (Table 6) of the plant extracts against the organism is an indication that methanol and ethyl-acetate extracts of *Azanza garckeana* had differing inhibitory activities against the various microorganisms studied. In agreement with this study, Lalitha and Jayanthi [40] indicated that biological activity of plant material depend largely on the type of solvent used in the extraction process. Therefore, selection of appropriate solvent for extraction of secondary metabolite for target organism inhibition is very vital t in the development of antimicrobial drug from plant origins.

Medicinal plants have been established to assuage pains and inflammation condition [41]. The present study revealed dose dependent reduction in the number of writhes in test animals. The percentages analgesia of 25.01%, 30.54 and 46.36% at the dose of 150, 300 and 600 mg/kg body weight respectively was observed while the standard acetyl salicylic acid (ASA) treatment caused percentages of analgesia 68.66% (Table 9). The egg albumin-induced rats' paw oedema are sensitive to cycloxygenase inhibitors and are used to evaluate the effect of non-steroidal anti-inflammatory agents [42]. In the present study the dose-dependent beneficial effects of methanol and ethyl-acetate extracts of *Azanza garckeana* on the inflammatory status observed in treated rats could be attributed to lowering TNF-α and IL-6 gene expression, which is correlated with the potent antioxidant property of the extracts thereby decreasing the activation of the redox-sensitive NF-κB [43].

Previous studies have also reported that plants rich in flavonoids, triterpenoids and saponins showed anti-inflammatory activity by inhibiting synthesis and release of inflammatory mediators, prostaglandins and polypeptide kinins [44]. Therefore, the presence of flavonoids and saponins in both methanol and ethyl-acetate extracts of *Azanza garckeana* may be responsible for its anti-inflammatory activities via the mechanism stated above. Furthermore, flavonoids and phenols have been documented for anti-inflammatory effects via inactivation of nuclear factor-kappa B and inhibiting the expression of COX-2, 5-LOX and MMP-9 and other inflammatory markers [44].

Rheumatoid arthritis is an autoimmune disorder, previous studies have indicated the role of oxidative stress in the pathogenesis of rheumatoid arthritis [45].. Interestingly, methanol extracts of *Azanza garckeana* exhibited increase inhibition of protein denaturation and increase membrane stabilization effect with increase extract concentration in both BSA and EGA models. In protein denaturation, tertiary and secondary protein structure are lost by heat, extrinsic stress, strong acid/base as well as organic solvent [46]. The mechanism of denaturation comprises variation in electrostatic, hydrophobic, hydrogen and disulphide linkage [47]. The plant extract produces a higher inhibitory percentage of protein denaturation with IC_{50} value of 310.44 μg/mL and 315.27 μg/mL in BSA and EGA assays respectively. This finding indicates the capability of the extract to ameliorate the denaturation of protein (albumin). The plant extract also exhibited an acceptable dose dependent membrane stabilization effects with IC_{50} value of 354.74 μg/mL and 349.57 μg/mL in BSA and EGA assays respectively (Table 7). The membrane stabilizing effect of the extract observed in this study, could be associated with its ability to interfere with the release of neutrophils lysosomal content [48]. Protective effect on erythrocyte lysis could possibly be acknowledged as an explicit indicator of anti-arthritic activity of the extracts [49].

Conclusion

This study lends pharmacological support to reported folkloric usage of *Azanza garckeana* (Goron Tula) in the treatment and management of several human disease. It is plausible that *Azanza garckeana* has been observed to exert significant antioxidants, antimicrobial, analgesic, anti-inflammatory, anti-arthritic and wound healing effect in experimental studies. Furthermore, methanol extract has been avowed to be most potent. Though, the exact mechanism of activities were not identified, its beneficial effects could possibly be correlated with the presence of important bioactive metabolites particularly phenols, alkaloids and flavonoids identified in the current study.

Acknowledgements
The authors would like to appreciate the technical staff of Biochemistry laboratory and animal holding unit of Federal University of Technology Minna, for their kind assistances.

Authors' contributions
The work is carried out in collaborations of all authors. All authors read and approved the final manuscript.

Funding
None.

Availability of data and materials
All data related to this manuscript are presented within the text.

Ethics approval
The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Biochemistry, IBB University Lapai, Lapai, Niger State, Nigeria. [2]Department of Biochemistry, Federal University of Technology Minna, Minna, Nigeria. [3]PhD Program for Cancer Molecular Biology and Drug Discovery, Taipei Medical University and Academia Sinica, Taipei 111, Taiwan. [4]Alex Ekweme Federal University Ndufu-Alike Ikwo, Abakaliki, Nigeria. [5]Senior Medical Officer, Hayok Medicare, Abuja, Nigeria.

Received: 2 January 2020 Accepted: 6 May 2020
Published online: 14 May 2020

References

1. World Health Organization (2018) This year's malaria report at glance. World Malaria Report. https://www.who.int/malaria/publications/world-malaria-report-2018/en/. Accessed 19 Nov 2019.
2. Toiu A, Mocan A, Vlase L, Pârvu AE, Vodnar AC, Gheldiu AM, Moldovan C, Oniga I. Phytochemical composition, antioxidant, antimicrobial and in vivo anti-inflammatory activity of traditionally used Romanian *Ajuga laxmannii* (Murray) Benth. (BNobleman's beard^ – Barba Împa˘ ratului). Front Pharmacol. 2018;9(7):1–16.
3. Umar SI, Lawal B, Mohammed BA, Obiekezie CI, Adewuyi AH, Babalola SB, Ariyeloye SD. Antioxidant and antimicrobial activities of naturally occurring Flavonoids from *M. heterophylla* and the safety evaluation in Wistar rats. Iran J Toxicol. 2019;13(4):39–44.
4. Onukogu SC, Tsado AN, Muhammad FM, Alawode RA, Suleiman A, Ojo OP, Oshevire RM, Mustapha A, Lawal B. In vitro antioxidants, antimicrobials and biochemical response of methanol leaf extract of Eucalyptus camaldulensis following sub-acute administration to rats. Saudi J Biomed Res. 2019;4(11):405–11.
5. Madaki FM, Kabiru AY, Ogbadoyi EO, Mann A, Mannesseh MJ, Lawal B. Antioxidant potential of Waltheria Indica methanol leaf extract in Trypanosoma Brucei Brucei infected mice. J Sci Technol Math Edu. 2019;15(1):295–309.
6. Lawal B, Shittu OK, Kabiru AY, Jigam AA, Umar MB, Berinyuy EB, Alozieuwa BU. Potential antimalarials from African natural products: a review. J Intercult Ethnopharmacol. 2015;4(4):318–43.
7. Umar SI, Ndako M, Jigam AA, Adefolalu SF, Ibikunle GF, Lawal B. Anti-plasmodial, anti-inflammatory, antinociceptive and safety profile of *Maytenus senegalensis* root bark extract on hepato-renal integrity in experimental animals. Comp Clin Pathol. 2019. https://doi.org/10.1007/s00580-019-02965-4.
8. Lawal B, Shittu OK, Oibiokpa IF, Mohammed H, Umar SI, Haruna GM. Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum. J Acute Dis. 2016;5(4):296–301.
9. Lawal B, Ossai PC, Shittu OK, Abubakar AN. Evaluation of phytochemicals, proximate, minerals and anti-nutritional compositions of yam Peel, maize chaff and bean coat. Inter J Appl Biol Res. 2014;6(2):01–17.
10. Manandhar S, Luitel S, Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J Trop Med. 2019;2019:1895340, 5 pages. https://doi.org/10.1155/2019/1895340.
11. Ibrahim AM, Lawal B, Abubakar AN, Tsado NA, Kontagora GN, Gboke GA, Berinyuy EB. Antimicrobial and free radical scavenging potentials of *N*-hexane and ethyl acetate fractions of *Phyllanthus Fraternus*. Niger J Basic Appl Sci. 2017;25(2):06–11.
12. Olugbodi JO, David O, Oketa EN, Lawal B, Okoli BJ, Mtunzi F. Silver nanoparticles stimulates spermatogenesis impairments and hematological alterations in testis and epididymis of male rats. Molecules. 2020;25:1063.
13. Kairupan F, Mantiri FR, Rumende RRH. Phytochemical Screening and Antioxidant Activity of Ethanol Extract of Leilem (Clerodendrum minahassaeTeijsm. & Binn) as an Antihyperlipidemic and Antiatherosclerotic Agent, CoSCI - PBBMIIOP Conf. Series: Earth and Environmental Science 217; 2019) 012016IOP Publishing. https://doi.org/10.1088/1755-1315/217/1/012016.
14. Ahmed RH, El Hassan MS, El Hadi HM. Potential capability of *Azanza garckeana* fruits aqueous extract on enhancement of iron absorption in Wistar albino rats. Int J Adv Res Biol Sci. 2016;3:245–50.
15. Alfred M. *Azanza garckeana* fruit tree: Phytochemistry, pharmacology, nutritional and primary healthcare applications as herbal medicine: a review. Res J Med Plant. 2017;11:115–23.
16. Glew RS, Vanderjagt DJ, Chuang LT, Huang YS, Millson M, Glew RH. Nutrient content of four edible wild plants from West Africa. Plant Foods Hum Nutr. 2005;60:187–93.
17. Akinnifesi FK, Kwesiga FR, Mhango J, Mkonda A, Chilanga T, Swai R. Domesticating priority for miombo indigenous fruit trees as a promising livelihood option for small-holder farmers in southern Africa. Acta Hort. 2004;632:15–30.
18. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT-Food Sci Technol. 2012;548–55(16):46.
19. Stanković MS. Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. Kragujevac J Sci. 2011;33:63–72.
20. Tease GE, Evans WC. Textbook of pharmacognosy. 12th ed. London: Tindalland and Co; 1983. p. 343–83.
21. Tsado AN, Lawal B, Ossai PC, Jagaba A, Gwadabe NK, Jiya AG, Umar AM, Oladunjoye J. O. Antioxidants and antimicrobial activities of methanol extract of *Newbouldia laevis* and *Crateva adansonii*. J Pharmacy Allied Health Sci. 2016;6:14–9.
22. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64:711–3.
23. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–200.
24. Halliwell B, Gutteridge JMC. Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Lett. 1981;128:347–52.
25. Oyaizu M. Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44:307–15.
26. Shilpa K, Chacko N, Shetty P. Investigation of anti-arthritic activity (*in- vitro* models) of *Hibiscus hispidissimus* Griffith. J Phytopharmacol. 2018;7(1):60–5.
27. Anosike CA, Onyechi O, Lawrence US. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum)*. DARU J Pharmaceutical Sci. 2012;20(76):1–7.
28. Koster R, Anderson M, Debeer EJ. Acetic acid method of analgesic screening. Current Opinion on Immunology. 1959;18:412–9.
29. Winter CA, Risley EA, Nuss GV. Carrageenin induced oedema in hindpaw of rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;3:544–7.
30. Sasidharan S, Logeswaran S, Latha LY. Wound healing activity of *Elaeis guineensis* leaf extract ointment. Int J Mol Sci. 2012;13:336–47. https://doi.org/10.3390/ijms13010336.

31. Sakha H, Rejila H, Shilpa S, Shreeya A, Dinesh D, Srijana T, Kamil P. Antimicrobial activity of Ethanolic extract of medicinal plants against human pathogenic Bacteria. TUJM. 2018;5(1):1–6.
32. Otang WM, Grierson DS, Ndip RN. Phytochemical studies and antioxidant activity of two south African medicinal plants traditionally used for the Management of Opportunistic Fungal Infections in HIV/AIDS patients. BMC Complement Altern Med. 2012;12:43.
33. Harborne JB. New naturally occurring plant polyphenols. In: Scalbert A, editor. Polyphenolic phenomena. Paris: INRA; 1993.
34. Hakkim FL, Shankar CG, Girija S. Chemical composition and antioxidant property of holy basil (Ocimum sanctum, L.) leaves, stems, and inflorescence and their in vitro callus cultures. J Agric Food Chem. 2007;55:9109–17.
35. Krishna-Kumar HN, Navyashree SN, Rakshitha HR, Chauhan JB. Studies on the free radical scavenging activity of Syagrus romanzoffiana. Int J Pharm Biomed Res. 2012;3:81–4.
36. Lawal B, Shittu OK, Ossai PC, Abubakar AN, Ibrahim AM. Antioxidant activities of Giant African snail (*Achachatina maginata)* Haemolymph against CCl_4- induced hepatotoxicity in albino rats. Brit J pharma res. 2015;6(3):141–54.
37. Mshelia EH, Watirahye EM, Maigari AU, Yohanna C, Ismail F. Cytotoxicity and antioxidant activity of stem bark extracts of *Azanza garckeana* (kola of Tula). Eur J Pure Applied Chem. 2016;3:16–24.
38. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005;100:80–4. https://doi.org/10.1016/j.jep.2005.04.025.
39. Mutindi MV. Phytochemical investigation of *Harrisonia abyssinica* and *Thespesia garckeana* for antiplasmodial and antimicrobial compounds. M.Sc. Thesis. Nairobi: University of Nairobi; 2014.
40. Lalitha TP, Jayanthi P. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms. Asian J Plant Sci Res. 2012;2:115–22.
41. Jigam AA, Mahmood F, Lawal B. Protective effects of crude and alkaloidal extracts of *Tamarindus indica* against acute inflammation andnociception in rats. J Acute Dis. 2017;6(2):78–81.
42. Wu SB, Dastmalchi K, Long C, Kennelly EJ. Metabolite profiling of Jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. J Agric Food Chem. 2012;60:7513–25.
43. Illesca P, Valenzuela R, Espinosa A, Echeverría F, Soto-Alarcon S, Ortiz M, Videla LA. Hydroxytyrosol supplementation ameliorates the metabolic disturbances in white adipose tissue from mice fed a high-fat diet through recovery of transcription factors Nrf2, SREBP-1c, PPAR-γ and NF-κB. Biomed Pharmacother. 2019;109:2472–81.
44. Clark RB. The role of PPARs in inflammation and immunity. J Leukoc Biol. 2002;71:388–400.
45. Shailasree S, Ruma K, Kini KR, Niranjana SR, Prakash HS. Potential anti-inflammatory bioactives from medicinal plants of Western Ghats, India. Pharmacogn Commun. 2012;2:2–12.
46. Bauerová K, Bezek A. Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis. Gen Physiol Biophys. 1999;18:15–20.
47. Prasad SB. Yashwant, Aeri V. in vitro anti-inflammatory activity of *Raupya (Silver) Bhasma*. J Chem Pharm Res. 2013;5:194–7.
48. Grant NH, Alburn HE, Kryzanauskas C. Stabilization of serum albumin by anti-inflammatory drugs. Biochem Pharmacol. 1970;19:715–22.
49. Alamgeer AMU, Hassan UA. Anti-arthritic activity of aqueous-methanolic extract and various fractions of *Berberis orthobotrys* Bien ex Aitch. BMC Complement Altern Med. 2017;17:371. https://doi.org/10.1186/s12906-017-1879-9.

Publisher's Note