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Original Research Article

Phytochemical screening and antimicrobial activities of hexane extract of *Azanza garckeana*

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Abstract

The method of cold maceration was used in the extraction. The phytochemical screening of crude yields of the chemical constituent of *A. garckeana* showed that flavonoids, steroids, phlobatannins, alkaloids, saponins were found to be present. The results of the susceptibility studies revealed the most of the test organisms were susceptible to the extract except *E. coli, Candida krusei and Vancomycin resistant enterococci* with all zero diameter zone of inhibition. Significant inhibitory effects were recorded on *Fomitopisis pinicola*on the extract with a diameter zone of inhibition *between* 21mm, *Aspergillus fumigate* (21mm), *Staphylococus aureus* (21), *Methicilin resist* (20), *Proteus mirabilis* (20). The samples are highly active as confirmed by the bactericidal and fungicidal activities of the crude extracts. A.*garckeana* could therefore be of pharmacological importance.

Keywords: *Azanza garckeana,* bactericidal activities, fungicidal activities, phytochemicals.

INTRODUCTION

Medicinal plants form an important component of flora and are widely distributed in the world. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can result to the development of novel and safe medicinal agents. Medicinal plants are composed of some certain organic compounds called phytochemicals which produce definite physiological actions in the human body and these bioactive substances include but are not limited to tannins, alkaloids, terpenoids, steroids and flavonoids (Edoega *et al*., 2005). The development of pharmaceutical products necessitates an exhaustive investigation of medicinal plants to improve our knowledge about their biological activities and the phytoconstituents responsible for them (Jothy *et al.,* 2012). Furthermore, the need for comprehensive investigations in this area is more evident owing to the fact that only a limited number of medicinal plant species have received complete scientific inspection (Mazumder *et al.,* 2008).

Drug resistance in both humans and animals is on the increase and it has become necessary to screen medicinal plants which can replace the synthetic drug and as they are biodegradable, safe, and less toxic. To the best of our knowledge little or no work has been done on the plant *Azanza garckeana* in Gombe state, Nigeria. This work is designed to enrich the available scientific data on the phytochemistry and antimicrobial activities of *A. garckeana* leaves. This paper reports the phytochemistry and antimicrobial activities of *H barteri* leaf extracts on some bacterial and fungal isolates.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material *Azanza garckeana* (Goron Tula) fruits were obtained from a local market in Tula Kaltungo local government area of Gombe state, Nigeria, in the month of February 2021.The plant was identified and authenticated by a botanist in the department of Biological sciences, Federal University Wukari, Wukari. Taraba State, Nigeria.

Preparation of Plant Material

The *Azanza garckeana* fruit was first of all deseed and the pulp(s) was air-dried at room temperature at about (37^0C), the pulp was pulverized using mortar and pestle to give fine powder and sieved using a sieving material whose mesh is 0.8mm size in diameter. Pulverized Fruits pulp(s) (172 g) were defatted with n- hexane for 72 hours by cold maceration methods, Extract was filtered and excess volume reduced using rotary evaporator, and evaporated to constant weight in fume cupboard before subsequent analysis.

Phytochemical Screening

Phytochemical examinations were carried out for the extract using standard procedures to identify the constituents. Qualitative analysis of the crude extract were carried out as described by (Ushie and Adamu, 2013, Sagayaraj *et al*., 2015, Ushie *et al.,* 2018 and Ushie *et al.,* 2019) to identify the presence of the classes of Phytochemicals (alkaloids, flavonoids, tannins, saponins, steroids and phenols).

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test

Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes indicates the presence of saponins.

Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of tannins

A small quantity or the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue black or brownish green indicate the presence of tannins.

Detection of Phenols

To 1ml of leaf extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for phlobatannins

A portion of each extract was boiled with 1% aqueous HCl. The solutions were observed for a red deposit of precipitate taken as evidence for the presence of phlobatannins.

Test for steroids

5 drops of concentrated H_2SO_4 was added to1ml of each extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts.

Antimicrobial Analysis of the Crude Extracts of *Azanza garckeana*

The antimicrobial activities of the methanol, ethyl-acetate and hexane extracts were determined using some pathogenic microbes obtained from the Department of medical microbiology Ahmadu Bello University, Zaria. The extracts (0.5geach) was weighed and dissolved in 10 ml of Dimethylsulphoxide (DMSO) to obtain a concentration of 5mg/ml. An agar well diffusion method was used for screening the extracts. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer's instructions sterilized at 121°c for 15 minutes, poured into sterile petri dishes and were allowed to cool and solidify. The sterilized medium was seeded with 0.1ml of the standard 2ipette2s of the test microbe. The 2ipette2s was spread evenly over the surface of the

medium by the use of a sterile swab. By the use of a standard sterile cork borer of 6mm in diameter, a well was cut at the centre of each inoculated medium. 0.1ml of the solution of the extract of the concentration of 50mg/ml was then introduced into the well on the inoculated medium. Incubation was made at 37°c for 24h after which the plates of the media were observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result recorded in millimetres.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentrations of the extracts were determined using the tube dilution method as outlined by (Onyeagba *et al.,* 2004). Set of eleven sterile test tubes were used. The tubes were labeled in the sequence with the following the dilution 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and control. 0.5ml of the Muller Hinton broth was 3ipette into the first tube (1:2 tubes). The solution was mixed thoroughly in the first tube by aspiration. Formation of bubbles was avoided. The diluted extract (0.5ml) was transferred to the second tube and this process was prepared until the highest dilution was obtained. The extract was not added to the control. 0.5ml of the diluted extract was discarded from the last tube. The bacteria were then dispersed in the broth culture by rotating the tube between your palms for at least 25 times.0.5ml culture was transferred to each tube including the control tube. This was mixed well after addition of the culture. The optical density was measured at 540nm. The tubes were incubated at 37^{o}C for 24hrs. The MIC value was taken at the least concentration of the extract showing no visible growth. The Minimum Bactericidal Concentrations were determined by assaying the test tube context of the MIC determinations. Aloopful of the content of each tube was inoculated by streaking on a solidified nutrient agar which it was observed for microbial growth. The lowest concentration of the sub culture showing no growth was considered as Minimum Bactericidal Concentrations. The same procedures were used to determine Minimum Fungicidal Concentration (MFC) for fungi but the tubes were incubated for 48hours at 37^{o}C.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Qualitative analysis of the crude extracts were carried out as described by (Sofowora, 1993; Edeoga *et al*., 2005; Tiwari, *et al*., 2011; Ushie and Adamu, 2013 and Sagayaraj *et al*., 2015) to identify the presence of the classes of Secondary Metabolites (alkaloids, flavonoids, tannins, saponins, steroids and phenols).

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

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A small quantity or the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue black or brownish green indicate the presence of tannins.

Detection of Phenols

To 1ml of leaf extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for phlobatannins

A portion of each extract was boiled with 1% aqueous HCl. The solutions were observed for a red deposit of precipitate taken as evidence for the presence of

Table 1. Extract Yield Percentage of hexane extract of *Azanza garckeana* fruit.

Sample Weight (g)	Extract Weight(g)	Percentage Yield %
172	0.4	0.23

Table 2. Qualitative phytochemical screening of hexane extract of *Azanza garckeana* fruit.

S/N	Phytochemicals	Tests	Hexane Extract
1	Flavonoids	Extract + NaOH	+
		Extract +Lead acetate	+
2	Alkaloids	Extract + Mayer	+
		Extract +wagner	+
3	Phlobatannins	Extract +2% HCl	+
4	Steroids	Extract + H_2SO_4	+
5	Saponins	Froth test	+
		Foam test	+
6	Tannins	Extract +H_2O + $FeCl_3$	+
7	Phenols	Extract + H_2O + $FeCl_3$	+

phlobatannins.

Test for steroids

5 drops of concentrated H_2SO_4 was added to1ml of each extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts.

RESULTS AND DISCUSSION

The yield percentage of *Azanza garckeana* was determined and shown in Table 1 indicating the n-hexane extracts of pulp gave a recovery percentage 0.25%. The phytochemical screening of crude yields of the chemical constituent of *A. garckeana* showed that flavonoids, steroids, phlobatannins, alkaloids, saponins were found to be present in the hexane extract. Hence, *Azanza garckeana* can be used as is a non-toxic and can they generate physiological responses in animals that consume them (McDevitt *et al*., 1996). The presence of tannin in the medicinal plant suggests the ability of these plants to play key roles as antifungal antidiarrheal, antioxidant and antihemorrhoidal agent (Asquith and Butter, 1986). *Azanza garckeana* have muscle relaxant property and can be utilized for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). Since alkaloids were detected in the Hexane extract. Alkaloids have been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine andanti-hypertensive anti-fungal, anti-inflammatory, antifibrogenic effect (Ghosal *et al.,* 1996). Alkaloids in plants are used in medicine as anaesthetic agents (Herourat *et al*., 1998). Flavonoids were detected in the hexane extract. The result revealed the presence of flavonoid in the extract. Hence, *A. garckeana* can be use to modifies the body's reaction toallergens, virus and caranogens. It has been reported to show anti-inflammatory, antifungi, antibacterial and antimicrobial activities based on the literature (Cushnie and Lamb 2005). (Table 2 and 3)

Azanza garckeana is important in pharmacy due to the presence of steroidal compounds which has a relationship in sex hormones (Okwu, 2001). Saponins were found to be present in the methanol extract and acetone extract, when the procedure for froth test was carried out. The presence of saponins in the seeds can be useful in treating inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness (Rita *et al*., 2015). Also in nature, saponins appear to act as antibiotics that protect plants from microbes (Opara *et al*., 2019). Phenols are present in the extracts of *A. garckeana*, thus can normally be involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as causative to plants colours. They are ubiquitous in all plant organs and are therefore an integral part of the human diet (Dai and Mumper 2010). Also, phenolic compounds, can inhibit the absorption of amylase in the treatment of carbohydrate absorption, such as diabetes (Sales *et al*., 2012).

The results of the susceptibility studies revealed that

Table 3. Antimicrobial Results of *Azanza garckeana* Crude Extract.

Microorganism	Hexane Extracts (mg/ml)	Zone of Inhibition (mm)		
		Sparfloxacin	Fluconazole	Fulcin
Methicillin resistant Streptococci	20	35	0	0
Vancomycin *resistenterococci*	0	0	0	0
Staphylococus aureus	21	31	0	0
Streptococus pyogens	0	30	0	0
E. coli	0	34	0	0
Klebsiella pneumonia	20	0	0	0
Proteus mirabilis	20	31	0	0
Candida albicans	0	0	34	0
Candida krusei	0	0	32	32
Aspergillus fumigates	21	0	0	32
Fusarium oxysporum	20	0	25	27
Fomitop sispinicola	21	0	0	31

almost all organisms showed excellent activity in the extracts except *E. coli, Candida krusei and* Vancomycin resistant enterococci all with zero diameter zone of inhibition. Significant inhibitory effect were recorded on *Fomitop sispinicola* on the three extracts with a diameter zone of inhibition of 21mm hexane extract, *Aspergillus fumigates* (21mm) and *Staphylococus aureus* (21mm) respectively. This is an indication that *A. garckeana* can provide a lead to cure to diseases associated with the tested bacteria such as endocarditis, urinary tract infection, renal diseases, liver disease, skin infection, pneumonia, bone and joint infections, etc. Medium inhibitory effects were recorded for *Methicillin resist* (20mm) for the hexane crude extract. The hexane crude extract of A. *garckeana* sample exhibited good activity on *Proteus mirabilis* with a diameter zone of inhibition of 25mm, an indication that *A. garckeana* extract can provide cure for diseases caused by this microbes such as complicated urinary tract infections (UTIs). These findings confirm the species antibacterial potential and its usefulness in the treatment and management of abscesses, syphilis, gonorrhea and sexually transmitted diseases respectively. There was no activity for the n-hexane crude extract of *A. garckeana* on *Candida albicans* and *candida krusei* with zero diameter zone of inhibition.

CONCLUSION

The presence of several compounds in the crude extracts of *Azanza garckeana* sample indicates its several pharmacological importances and despite its varying chemical composition, it is highly biologically active as confirmed by the bactericidal and fungicidal activities of the crude extracts. Hence the study describes the potential use of *Azanza garckeana* as a source of natural ethno medical supplements.

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