Evaluation of the Antitussive and Expectorant Activities of Fruit Extract and Fractions of Azanza Garkeana.

Chukwuebuka Chukwuezugolum Onwuzuligbo[1*], Chiamaka Hansa Ibezimako[1], Nnamdi Peter Odionyenma[1], Akuchukwu Jedidiah Onwuzuligbo[2] and Chukwunonso Chukwudike Onwuzuligbo[3].

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State

[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State.

[3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State

***Corresponding Author**

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ABSTRACT

Azanza garkeana is a medicinal fruit traditionally used in African ethnomedicine for treating respiratory ailments such as cough and sore throat. This study evaluated the phytochemical composition, acute toxicity, antitussive, and expectorant activities of its ethanol extract and solvent fractions. The fruits were extracted with ethanol and fractionated into n-hexane, ethyl acetate, butanol, and aqueous fractions. Phytochemical screening revealed the presence of flavonoids, tannins, glycosides, steroids, and triterpenoids. Acute toxicity testing in mice showed no mortality up to 5000 mg/kg, indicating a high safety margin. In the ammonia-induced cough model, the crude extract and butanol fraction produced significant dose-dependent reductions in cough frequency, with the 500 mg/kg butanol fraction showing effects comparable to codeine ($p < 0.05$). Similarly, in the expectorant assay, the butanol and ethyl acetate fractions enhanced tracheal phenol red secretion, which is an indication of increased mucus clearance. These findings suggest that *A. garkeana* possesses potent antitussive and expectorant activities, likely attributable to its flavonoids, tannins, and glycosides, and support its traditional use in the management of respiratory disorders.

Keywords: Azanza garkeana, phytochemistry, acute toxicity, antitussive, expectorant, respiratory disorders

INTRODUCTION

Coughing is a common respiratory symptom that affects millions of people worldwide. It can be caused by various factors, including infections, allergies, and environmental irritants [1]. In some cases, coughing can be severe and persistent, leading to discomfort, sleep disturbances, and decreased quality of life [2]. Respiratory tract disorders, particularly cough and mucus overproduction, remain among the most common health complaints globally and are major contributors to outpatient consultations and self-medication [1]. Cough is often managed with synthetic antitussives such as codeine and dextromethorphan, while expectorants like guaifenesin are employed to enhance mucus clearance. However, these conventional drugs are associated with adverse effects including sedation, dependence, and limited efficacy in chronic conditions [3]. This has stimulated renewed interest in plant-based therapies, many of which are used traditionally for respiratory ailments and are generally considered safer, more affordable, and accessible in resource-limited settings [4].

Traditional medicine has long employed various plant extracts to alleviate cough symptoms. One such plant is Azanza garckeana (commonly known as Gorontula), which has been used in folk medicine for its perceived antitussive (cough-suppressing) properties [5]. Azanza garckeana is the botanical name for Gorontula, a multipurpose edible fruit with a sweet and chewy nut often referred to as 'African Chewing Gum'. Known as

snot apple, Silky Kola, Azanza, tree hibiscus or wild hibiscus in English. A. garckeana is a valuable edible indigenous fruit tree species. In Nigeria A. garckeana is found in Tula area of Kaltungo Local Government Area of Gombe State [6]. Gorontula is a 'miracle fruit' packed with loads of healthy and sexual benefits [6, 7]. So far, it has been used to treat chest pains, cough, liver problems, prevention of cancer and menstrual problems amongst other diseases [7, 8]. Despite its widespread use, there is a dearth of scientific evidence supporting the antitussive efficacy of Gorontula. Most studies on the plant's medicinal properties have focused on its aphrodisiac, antimicrobial, anti-inflammatory, and antioxidant activities, with little attention paid to its potential antitussive effects [5].

The present study aims to bridge this knowledge gap by evaluating the antitussive properties of *Azanza garckeana*. Specifically, this research aims to evaluate the plant's ability to suppress coughing in a controlled laboratory setting [9].

MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Material

The fruits of Azanza garkeana were collected in large quantities from a vendor in Owerri, Imo state. The fruits where in good conditions and fresh so as to ensure a high yield of the extract.

2.1.2 Chemicals

Chemicals and experimental reagents used include: Ethanol (JHD, China), Butanol (JHD, China), n-Hexane (JHD, China), Ethyl acetate (JHD, China). All solvents/ reagents purchased were of analytical grade. All laboratory reagents were freshly prepared and freshly distilled water was used when required.

2.1.3 Equipment

Weighing balance (OAHUS, Poland), Beakers, measuring cylinder, sample tubes, spatula, water, separating funnel, Conical flask, permanent marker, Tripod stand, water bath, muslin cloth, extraction chamber, Spectrophotometer (Model 752, China), rotary evaporator (RE300 Model, United Kingdom).

2.1.4 Animals

Adult Swiss albino mice (25 – 30 g) of both sexes were used for this study. The animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria. The animals were maintained on standard laboratory animal conditions and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to water ad libitum. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and approved by the Nnamdi Azikiwe University Animal Ethical Committee with approval number NAU/AREC/2025/0044 and NAU/AREC/2025/0046

2.2 Method

2.2.1 Preparation of the fruit sample

The fruits were collected and washed gently with water and dried in a room temperature. It was then pulverized into a coarse form using a milling machine.

2.2.2 Selection of solvent

Appropriate solvent was chosen based on the nature of the target compounds and their solubility. Ethanol was used in this case. The polarity of the compounds wished to be extracted is also considered. A wide range of varying polarities require a mixture of solvents or sequential extraction process with solvents of different polarities.

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2.2.3. Extraction:

2.2.3.1 Plant extraction and fractionation

Two and half kilogram of the pulverized leaves *A. garkeana* extracted by cold maceration in 7.5L of ethanol for 72 h with intermittent shaking. The resulting solution was filtered, and the filtrate concentrated to dryness *in vacuo* using rotary evaporator (RE300 Model, United Kingdom) at 40°C. Then 200 g of the extract was dissolved in 400 ml of water and subjected to liquid-liquid partition successively and exhaustively with n-hexane, ethyl acetate and n-butanol in increasing order of polarity. Fractions soluble in these solvents were concentrated *in vacuo* using rotary evaporator to obtain the n-hexane fraction (HF), ethyl acetate fraction (EAF) and n-butanol fraction (BF). The remaining fraction was the water fraction (WF). The plant extract and fractions were stored in refrigerator between 0-4°C.

2.2.3.2 Aqueous extract

200g of the coarse pulverized sample was boiled in 1.5litre of water for 5mins. It was allowed to cool and was sieved using porcelain cloth. It was further sieved with No 1 Whatman filter paper. The filtrate was concentrated using water bath at 50^0c. The crude extract was stored in the refrigerator for use.

2.3 Phytochemical Screening of *Azanza garkeana*

Qualitative chemical analysis for chemical constituents of Azanza garckeana fruits were determined using the method of odebiyi and sofowora [10]; Harborne [11] and Evans [12]. With some modifications.

2.4 Acute toxicity test

The acute toxicity test was performed using the method of Lorke [13]. A total of 13 adult albino rats were used, and it was performed in two phases. Phase I, a total of 9 rats were used and they were grouped into 3 groups of 3 rats each. Group I received 10mg/kg crude extract orally, Group II received 100mg/kg crude extract orally, Group III received 1000mg/kg crude extract orally. The animals were constantly monitored for the next 1hr, intermittently for the next 3hrs and finally after 24hrs for signs of behavioral changes and mortality. From the result of the phase I, the second phase was carried out. In the second phase, a total of 4 rats were used. They were grouped into four groups of one rat per group. Group I received 1600mg/kg orally, group II received 2900mg/kg orally, group III received 5000mg/kg orally, group IV received 5000mg/kg orally. The animals were monitored for behavioral changes.

2.5 Procedure for Anti-tussive study test

The Anti-Tussive study of *Azanza garckeana* was carried out according to the method employed by Awe *et al* [14]. A total 70 of adult albino mice was used. They were grouped into groups of five mice per group. Group 1 received 10mg/kg of distilled water; group 2 received 25mg/kg codeine phosphate; group 3, 4 and 5 received 100mg/kg, 250mg/kg and 500mg/kg crude extract respectively; group 6 and 7 received 250mg/kg and 500mg/kg n-hexane fractions respectively; group 8 and 9 received 250mg/kg and 500mg/kg ethyl acetate fractions respectively; group 10 and 11 received 250mg/kg and 500mg/kg butanol fractions respectively; and group 12, 13 and 14 received 100mg/kg, 250mg/kg and 500mg/kg water extract respectively. All the administration was given orally. An hour after post treatment, each mouse was placed in a 1000ml diameter special chamber embedded with cotton wool and exposed to 25% ammonia solution for 45secs. The mice were taken out and put in a chamber with an opening cut the top and cough frequency was courted for 5mins

2.6 Procedure for Expectorant test

The expectorant test was carried out according to the method of Zhang *et al* [15]. A total of 70 adult mice was used. They were grouped into… groups of 5mice per group. Group 1 received 10mg/kg of distilled water; group 2 received 25mg/kg codeine phosphate; group 3, 4 and 5 received 100mg/kg, 250mg/kg and 500mg/kg crude extract respectively; group 6 and 7 received 250mg/kg and 500mg/kg n-hexane fractions respectively; group 8 and 9 received 250mg/kg and 500mg/kg ethyl acetate fractions respectively; group 10 and 11 received 250mg/kg

and 500mg/kg butanol fractions respectively; and group 12, 13 and 14 received 100mg/kg, 250mg/kg and 500mg/kg water extract respectively. All the administration were given orally. After 30minutes post treatment, 0.2ml/20g of 5% phenol red in physiological saline was administered orally to the mice. Then 30minutes after the administration the animals were sacrificed by cervical dislocation without damaging the trachea. The trachea was removed between the thyroid cartilage and the main Sten bronchi after which it was sonicated for 10mins. A 0.1ml of 1M NaOH solution was added. The absorbance was read at 546nm using spectrophotometer.

2.7 Statistical analysis

Results were presented as mean ± Standard error of mean (SEM). The analysis of variance in the outcome of the treatment (one way ANOVA) was done using Statistical Package for Social Science (SPSS, version 20). Multiple comparisons for post hoc analysis was done using Turkey's test. The differences between the treatment groups were determined by multiple comparisons of mean ranks for all groups. In all cases, a probability error of less than 0.05 was selected as the criterion for statistical significance. Calculation of fifty percent inhibitory concentration (IC50) of the extracts and fractions was carried out using regression equation in Microsoft Excel, 2007.

RESULTS

3.1 Qualitative Phytochemical Analysis

Table 1 below shows the results of the qualitative phytochemical analysis; showing the different phytochemicals present in the respective solvent extracts.

Table 1: Result of Qualitative Phytochemical Analysis

Phytochemical class	Crude extract	N-Hexane	Ethyl acetate	Butanol
Alkaloids	-	-	-	-
Flavonoids	+	-	+	+
Reducing sugars	+	-	+	+
Saponins	-	-	-	-
Proteins	-	-	+	-
Tannins	+	-	+	+
Amino Acids	-	-	+	-
Steroids	+	+	+	-
Triterpenoids	-	+	+	-
Glycosides	+	-	+	+

3.2 Acute Toxicity Studies

Table 2 shows the acute toxicity studies, indicating how safe or otherwise the extracts from the plant are

TABLE 2: Result of Acute Toxicity Studies

PHASE 1	DOSE (ml/kg)	NUMBER OF DEATH
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3

PHASE 2	**DOSE (ml/kg)**	**NUMBER OF DEATH**
Group 1	1600	0/1
Group 2	2900	0/1
Group 3	5000	0/1

The oral LD50 was therefore estimated to be above 5000mg/kg in mice

3.3 Antitussive Activity

Table 3 and Fig. 1 below show the ability of the different solvents' extracts of the plant in suppressing cough episodes.

Table 3: Result of Antitussive Effect

Groups	Number of Cough
10ml/kg of distilled water	74.3±4.1
25mg/kg codeine	16±3.21
100mg/kg crude	27.7±2.03
250mg/kg crude	13±3.46
500mg/kg crude	8.67±1.86
250mg/kg N-hexane	39±4.48
500mg/kg N-hexane	29.3±0.882
250mg/kg ethyl acetate	33.3±0.882
500mg/kg ethyl acetate	19.3±2.96
250mg/kg butanol	17.3±1.45
500mg/kg butanol	5.67±2.33
100mg/kg water extract	26.3±2.6
250mg/kg water extract	14±2.08
500mg/kg water extract	10±0.3

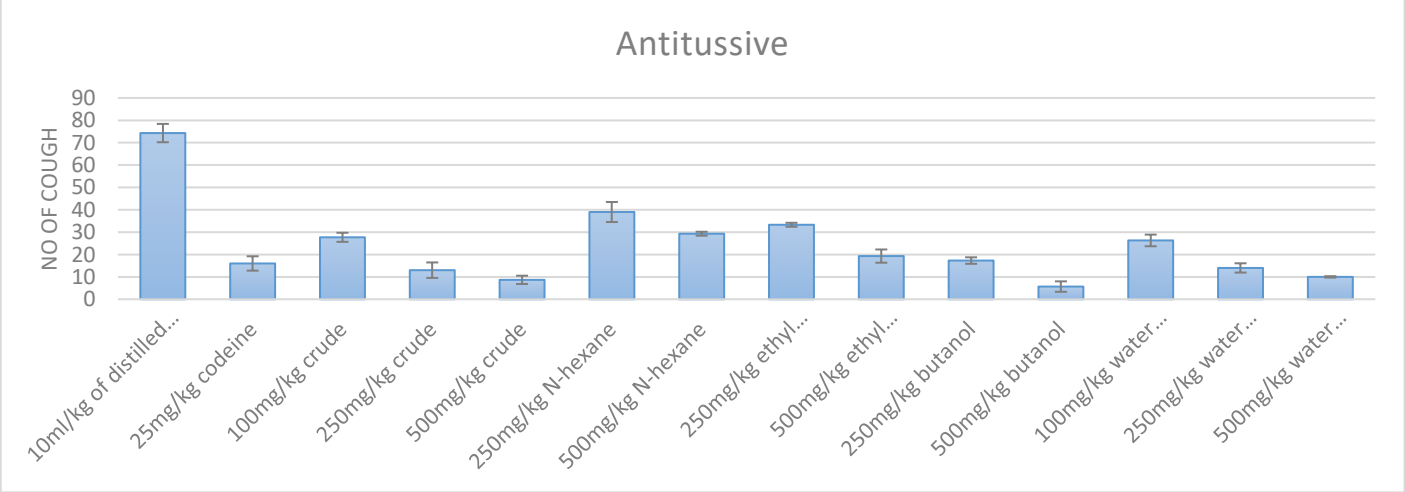

Fig 1. Bar chart of antitussive effect showing the graph of number of cough vs. dose

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3.4 Expectorant Activity

The expectorant activities of the different solvents' extracts are as shown in Table 4 and figure 2 below.

Table 4: Result of Expectorant Activity

Groups	Mean absorbance
10ml/kg of distilled water	0.274±0.0056
25mg/kg codeine	0.142±0.0031
100mg/kg crude	0.224±0.0061
250mg/kg crude	0.19±0.0043
500mg/kg crude	0.146±0.0075
250mg/kg N-hexane	0.215±0.0032
500mg/kg N-hexane	0.181±0.0046
250mg/kg ethyl acetate	0.128±0.002
500mg/kg ethyl acetate	0.188±0.0027
250mg/kg butanol	0.142±0.0064
500mg/kg butanol	0.117±0.0038
100ml/kg water extract	0.221±0.0019
250ml/kg water extract	0.188±0.00266
500ml/kg water extract	0.144±0.0047

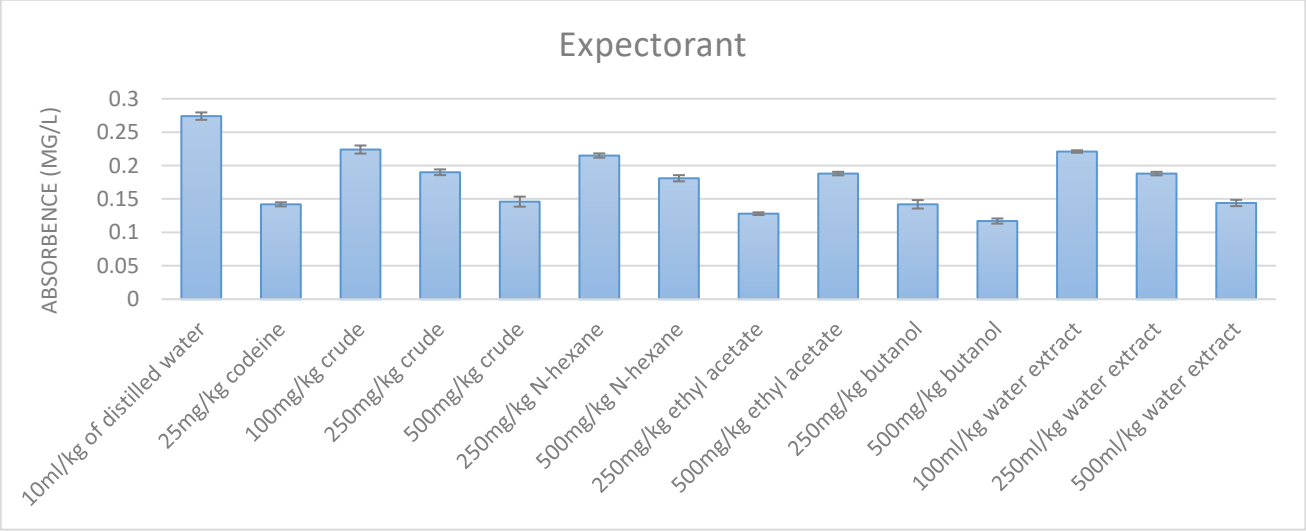

Fig 2. Bar chart of antitussive effect showing the graph of absorbance vs. dose

DISCUSSION

The present study investigated the phytochemical composition, acute toxicity, antitussive, and expectorant activities of *Azanza garkeana* fruit extracts and solvent fractions. Phytochemical screening revealed the presence of flavonoids, tannins, steroids, glycosides, and reducing sugars in the ethanol, ethyl acetate, butanol, and aqueous extracts, while n-hexane extracts contained predominantly steroids and triterpenoids. The absence of alkaloids and saponins in all fractions is consistent with previous reports on the secondary metabolites of *A. garkeana* [7]. The abundance of flavonoids and tannins is noteworthy, as these phytochemicals have been

implicated in cough suppression and airway soothing effects due to their antioxidant, anti-inflammatory, and mucosal protective properties [7, 16].

The acute toxicity studies revealed no mortality up to the limit dose of 5000 mg/kg, suggesting that *A. garkeana* possesses a high safety margin when administered orally. According to the OECD classification, extracts with LD_{50} values greater than 5000 mg/kg can be considered practically non-toxic [17]. This finding supports its traditional use as an edible fruit and medicinal remedy in African ethnomedicine [7]. The lack of behavioral abnormalities or toxic symptoms further underscores its tolerability, aligning with earlier toxicity studies of plant-derived remedies rich in polyphenols [4].

The antitussive evaluation demonstrated that the crude ethanol extract and its solvent fractions significantly reduced cough frequency induced by ammonia exposure in mice in a dose-dependent manner. At the highest doses tested (500 mg/kg), the crude extract and butanol fraction produced cough suppression comparable to the reference drug, codeine. These findings suggest that the bioactive principles responsible for antitussive activity may be enriched in the butanol fraction, which contained flavonoids, tannins, and glycosides. Flavonoids, in particular, are known to suppress cough effects through their ability to reduce airway inflammation, inhibit histamine release, and modulate neuronal pathways involved in cough reflexes [18, 19].

In addition, the expectorant assay revealed that treatment with the extracts increased phenol red secretion in the trachea, with the butanol and ethyl acetate fractions producing effects comparable to codeine. Enhanced secretion of phenol red is an indication that respiratory tract fluid increased, which facilitates mucus clearance and relieves obstruction of airway [15]. The observed expectorant effects can be linked to the presence of flavonoids and glycosides, which have been reported to stimulate mucus production and modulate airway smooth muscle tone [20]. The dual antitussive and expectorant properties observed in *A. garkeana* justify its folkloric use in managing cough, sore throat, and other respiratory conditions.

Collectively, these findings suggest that *Azanza garkeana* possesses significant bioactive potential as a natural remedy for respiratory ailments. The butanol fraction appears to harbor the most active compounds, making it a candidate for future isolation and characterization studies. Furthermore, the safety profile of the fruit extract enhances its therapeutic promise, especially in regions where synthetic antitussive drugs may be associated with side effects or limited accessibility. However, further studies including mechanistic investigations, chronic toxicity testing, and clinical validation are needed to fully establish its efficacy and safety in humans.

CONCLUSION

A. garkeana possesses promising therapeutic potential as a natural remedy for respiratory tract ailments. However, further studies are recommended to isolate and characterize the specific bioactive compounds, elucidate their mechanisms of action, and evaluate their safety and efficacy in long-term and clinical settings.

Compliance with ethical standards: All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and approved by the Nnamdi Azikiwe University Animal Ethical Committee with approval number NAU/AREC/2025/0044 and NAU/AREC/2025/0046

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