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Quantitative and Qualitative Phytochemical Screening of Aqueous and Ethanol Extracts of Indole Acetic Acid-Treated Okra Fruits.

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Abstract

Okra (*Abelmoschus esculentus* L.) is an important medicinal plant. The control and indole acetic acid-treated okra fruits ground powder was subjected to aqueous and ethanol extracts separately by the maceration method. The qualitative and quantitative analysis were confirmed in both aqueous and ethanol fruits powder extracts. The presence of tannins, saponin, flavonoids, alkaloids, terpenoids, and cardiac glycosides were ascertained in aqueous and ethanol fruits powder extracts. The quantitative results of the aqueous and ethanolic crude extracts of fruit powder revealed the total flavonoids content in ethanol extracts of the control and indole acetic acid-treated fruit powder in the range of 15.35 ± 3.00 to 20.79 ± 3.65 mg of QE/g dw. In comparison, the tannins and cardiac glycosides contents were in the range of 120.00 to 200.65 mg/100 g and 95.50 to 100.55 mg/100 g, respectively. The perethanol crude extract of indole acetic acid-treated fruit powder, and the values are 14.45 and 10.59%, centage yield of saponin and alkaloids was higher in the respectively. The present study results showed that okra might be a promising target for the phytochemicals exploitation for health benefit and as a substitute medicine.

Keywords: Okra, indole acetic acid, crude extracts, phytochemical constituents.

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Introduction

Various bioactive compounds are produced virtually in all parts of plants. They have medicinal value useful for human beings and animals, while the primary metabolites are essential to the plants [1]. These plants metabolites include cyanogenic glycosides, tannins, terpenoids, steroids, saponins, carotenoids, flavonoids, and alkaloids. Phytochemical constituents of a plant represent its medicinal values with effects on the human body. Hence, screening these bioactive compounds could help detect an important compound that could be applied as the sources of modern drugs [2]. Most bioactive compounds have antioxidant properties that ameliorate the damage caused by the reactive oxygen species (ROS) to the body tissues [3]. Various biotic and abiotic factors like climate change etc. affect plant growth and development, which invariably affect the quality of bioactive compounds present in the plants [4]. Plant hormones are organic molecules that positively interfere with plant growth and development [5]. Plant hormones concentration, type, and application time are essential factors for the accumulation and metabolism of the secondary metabolites, which in turn affect the essential oil content in plants [6].

Many vegetable crops have secondary metabolites with biological activities, which interact with various metabolic processes. One of these vegetables is okra (*Abelmoschus esculentus* (L.), which is an important vegetable crop grown mainly in the tropical or sub-tropical regions during summer and the rainy season [7]. Okra is widely grown in Africa, Asia, Southern Europe, and America [8].

It is produced in large quantities in various countries like India (3.5 million tons), Nigeria (0.73 million tons), Pakistan (0.12 million tons), Ghana (0.10 million tons), Egypt (0.08 million tons), and Benin (56 564 tons) [7]. Okra is a multipurpose crop as its pods, fresh leaves, buds, flowers, stems, and seeds have several uses. Its immature fruits, which are consumed as vegetables, can be used in salads, soups, stews, fresh or dried, fried, or boiled [9]. Besides, the plant has been used medicinally in the treatment of several disorders. Anti-cancer, antimicrobial, and hypoglycaemic activities of the plant are reported. The anti-ulcer activity of fresh fruits is recently reported [10]. it is a vegetable of high value due to its high nutritional importance [11], with a significant proportion of fiber that reduces intestinal sugar absorption [12]. Traditionally, every part of the okra plant is used for medicinal purposes, such as in the management of diuretic, cooling, aphrodisiac, antiseptic, and gonorrhea [10]. This study is aimed to determine the bioactive constituents of indole acetic acid-treated okra fruit.

Materials and Methods

Plant materials

From our previous study, the most effective and promising group of okra fruits treated with 0.4 mM IAA (indole acetic acid) and control fruits were used for this study [13]. The samples of the selected okra fruits were used for the phytochemical

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analysis using standard methods in the Nutritional and Industrial Biochemistry Department, University of Ibadan, Nigeria.

Preparation of Fruit Extracts

The control and indole acetic acid-treated okra fruits were made into powder for extraction after drying at room temperature, respectively. For aqueous extraction, fruit powder was dissolved into 2500 mL of distilled water in ratio 1:10, respectively. Stirred until it was viscous, it was then heated and boiled for about 10 min and then cooled for 10 min before the mixture was centrifuged at 1500 rpm for 10 min. The supernatant was decanted and stored at -80°C before transferring into the freeze dryer machine in order to get soluble powder, which was stored for the experiment.

For the ethanol extraction, the fruits powder was dissolved in a round bottom flask containing 75% ethanol in ratio 1: 10. The mixture was stirred using a magnetic stirrer at a temperature of 40°C for 3 hours. After this, they were decanted, sieved, and transferred into the rotary evaporator equipment to remove ethanol and concentrate the solution before transferring it into the freeze dryer machine to get soluble powder, which was stored for the experiment.

Okra Fruit Sample Qualitative Phyto-chemicals Analysis

Qualitative screening of the extracts was done by following the methods described by Parekh and Chands and Ejikeme *et al*. [14,15].

Test for Tannins

The method of Ejikeme *et al*. [15] was used for tannins analysis. Briefly, 0.5 g powder of each sample was separately boiled in a test tube containing 40 mL of water and then filtered. A few drops of 0.1% $FeCl_3$ was added. The brownish-green color developed confirmed tannins.

Test for Saponin

Each plant sample powder of about 0.5 g was separately boiled in 10 mL of distilled water in a test tube using a water bath and then filtered. The filtrate (5 mL) was mixed with 2 ml of distilled water and vigorously swirled to ensure persistent frothing. Olive oil of about 1-2 drops was added to the mixture and shaken vigorously, then the formation of emulsions observed confirmed saponins [14].

Test for Flavonoids

Flavonoids content was determined by using the method of Ejikeme *et al*. [15]. Briefly, a 1% solution of ammonia (NH_3) was mixed separately with 2 ml of the filtrate of extract, and then concentrated H_2S0_4 was added. A yellowish color developed, which later disappeared on standing confirmed flavonoids.

Test for Alkaloids

A 0.5 g crude extract of each plant sample was acidified in hot 8 mL of 1% HCl separately and then filtered. Ammonia and chloroform of about 5 mL were added to the filtrate of approximately 3 mL and then vortexed. Acetic acid (10 mL) was used to extract the chloroform part. The chloroform extracted was divided into two parts. Each portion dissolved with Mayer's reagent and Draggendorf's reagent, respectively. Reddish-brown precipitate with the two reagents, respectively, confirmed alkaloids [15].

Test for Cardiac Glycosides

The crude extracts of about 5 mL each were added to 1 mL of concentrated sulphuric acid,

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then with glacial acetic acid (2 mL), followed by one drop of ferric chloride ($FeCl_3$). A brown ring forming at the test tube edge confirmed the presence of cardiac glycosides.

Test for Terpenoids

The analytical procedure used was according to Parekh and Chands (2008) [14]. A 2 mL chloroform was mixed with 5 mL crude extract of each sample and then followed with 3 mL of concentrated H_2SO_4. A reddish-brown color developed, confirming the presence of terpenoids.

Test for Phlobatannins

Each plant sample powder of about 0.5 g was soaked in distilled water (30 L). A 10 mL of aqueous extract after 24 hours was added to the extraction and subjected to boiling in 1% HCl. Red precipitate deposit confirmed phlobatannins [15].

Okra Fruit Sample Quantitative Phyto-Chemicals Analysis

Tannin Content Determination

The quantitative determination of tannin was performed by Amadi *et al*. [16] method. A 1 g of each crude extract was added to 100 mL of distilled water in a conical flask and boiled gently using a water bath for 1 hour. It was then filtered into a 100 L volumetric flask using filter paper Whatman No 1. The mixture of 10 mL of saturated Na_2CO_3 solution and 5.0 mL Folin-Denis reagent was mixed into the distilled water (50 L). The 10 mL of the mixture was pipetted out into the 100 L conical flask for the color formation. A standard used was tannic acid at a concentration ranging from 0.2–1.0 mg/cm^3. The concentration of tannins was determined at 700 nm using a UV/VIS spectrophotometer.

$$Tannic\ acid\left(\frac{Mg}{100g}\right)=\left(\frac{C\times extract\ volume\times 100}{Aliquot\ volume\times weight\ of\ sample}\right) \quad (1)$$

Where C is the concentration of tannic acid.

Determination of Saponin Content

Saponin content was estimated by the method of Obadoni and Ochuko [17]. A 250 mL conical flask containing 5g of each extract and 100 mL of 20% aqueous ethanol. The mixture was heated in a water bath at 55 °C for more than three hours, coupled with continuous stirring. The water bath at 90° C was used to concentrate the extract to less than half of the original volume. To the concentrate in the 250 L separating funnel, 20 mL of diethyl ether was added and vigorously swirled to separate the aqueous and ethyl layers. This step was repeated twice, after which n-butanol (60 mL) was added and extracted twice with 5% sodium chloride (10 mL). The remaining solution was heated in a water bath for 30 min. after the sodium chloride solution had been discarded. Then, the solution was transferred into a crucible and oven-dried until a constant weight is achieved. The concentration of saponin was calculated as follows.

$$\%Saponin=\frac{Weight\ of\ saponin}{Weight\ of\ sample}\times 100 \quad (2)$$

Alkaloid Content Determination

Alkaloid concentration was measured by the method of Ejikeme *et al*. [15]. To 2.5 g of each extract of the sample in a 250 L beaker, 200 mL of 10% acetic acid in ethanol was added and allowed to stand for about 4 hours. A water bath was used to concentrate the extract to about 40 ml

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of the initial volume, and then 15 drops of concentrated ammonium hydroxide were added drop-wise for precipitation. After the sedimentation of the mixture for 3 hours, the upper layer was discarded, and the sediments were washed with 0.1 M of ammonium hydroxide (20 ml). Then the residue was filtered using Whatman No.1 filter paper. The residue was oven-dried, and the alkaloid concentration was calculated as follows.

$$\%Alkaloid = \frac{Weight\ of\ alkaloid}{Weight\ of\ sample} \times 100 \quad (3)$$

Flavonoid Content Determination

The colorimetric method of Xu and Chang [18] was used to determine the flavonoids concentration. Briefly, Crude extract of about 0.25 mL was added to distilled water (1.25 mL) in a test tube, and then 75 µL of a 5% $NaNO_2$ solution was added. After 360 secs, then the addition of 150 µL of a 10% $AlCl_3$ solution and allowed for some time before 0.5 mL of 1 M NaOH was added. Distilled water was added to the mixture to the required volume of 2.5 mL and mixed well. The absorbance reading was taken at 510 nm against the blank by using a UV/VIS Spectrophotometer. The standard used was quercetin, and the result obtained was expressed as micrograms of quercetin equivalents of dry weight (mg of QE/g dw).

Cyanogenic Glycosides Content Determination

The cyanogenic glycosides concentration was estimated by using the Amadi *et al*. [16] method. Briefly, to a 1 g of each sample in a 250 L round bottom flask, 200 L of distilled water was added, and allowed for about 2 hours for autolysis to occur. A 20 mL of 2.5% NaOH was added into the flask to ensure full distillation. After which an antifoaming agent (tannic acid) was added. To the distillate, The addition of 100 mL of cyanogenic glycosides, 6 M NH_4OH (8 mL), and 5% KI (2 mL) occurred, mixed thoroughly, and 0.02 M $AgNO_3$ was used for the titration against a black background using a microburette. The endpoint was indicated by turbidity. The cyanogenic glycosides concentration was estimated as follows.

$$Cyanogenic\ glycoside\left(\frac{mg}{100g}\right) = \frac{Titre\ Value\left(cm^3\right) \times 1.08 \times exact\ volume}{Aliquot\ volume\left(cm^3\right) \times sample\ weight\left(g\right)} \times 100 \quad (4)$$

Statistical Analysis

The data were presented as mean ± SD. The difference was considered statistically significant when $p < 0.05$.

Results and Discussion

Table 1 shows a qualitative assessment of aqueous and ethanol crude extracts of okra fruit powder samples. The results showed the presence of various phytochemical compounds like tannins, saponin, flavonoids, alkaloids, and cardiac glycosides. The tannins and flavonoids contents were significantly present in the crude extracts of both extraction media and with little or no phlobatannins and terpenoids.

Table 2 revealed the quantitative analysis of the okra fruit crude extracts. The results showed the quantity of the various phytochemical compounds. The total flavonoids content in ethanol extracts of control and indole acetic acid-treated fruit powder showed different results ranged from 15.35 ± 3.00 to 20.79 ± 3.65 mg of QE/g dw. In comparison, the tannins and cardiac glycosides contents

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ranged from 120.00 to 200.65 mg/100 g and 95.50 to 100.55 mg/100 g, respectively. The percentage yield of saponin and alkaloids was higher in the ethanol crude extract of indole acetic acid-treated fruit, and the values were 14.45 and 10.59%, respectively, as compared to the aqueous crude extract.

The bioactive compounds in plant species are responsible for their therapeutic activity. The qualitative and quantitative screening of control and indole acetic acid-treated okra fruit extracts were determined in this study. The bioactive compounds analysis and the confirmation of tannins, saponin, flavonoids, alkaloids, and cardiac glycosides in okra fruit extracts showed that okra has a potential for mitigating various diseases. The higher concentrations of these phytochemical constituents observed in the indole acetic acid-treated okra fruit powder extracts may be due to the ameliorative effect of indole acetic acid on environmental factors that disturb the metabolism of these bioactive constituents [4].

From the results, the higher concentrations of tannins and flavonoids observed in the crude extracts showed the medicinal potential of okra fruit. Osuntokun *et al*. [19] reported tannins as an important bioactive compound found in plant-based medicines. Tannins are used as an antioxidant in beverages [19] and also possesses antiviral, antitumor, and antibacterial activity [14]. Flavonoids have an antioxidant property with the ability to prevent tumors initiations, promotion, and progression [20] reported the association of flavonoids to the reduction of coronary heart disease. The saponins are used as an antioxidant in hyperglycaemia, hypercholesterolemia, weight loss, and cancer [21]. Akinyeye *et al.* [22] reported in his study that alkaloids have both antibacterial and anti-diabetic properties. All these data corroborate the importance of every part of okra for medicinal purposes.

Table 1: Qualitative phytochemicals analysis of aqueous and ethanol okra fruits powder extracts

Phytochemical constituents	Control fruit powder		IAA-treated fruit powder	
	Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract
Tannins	++	+++	+++	+++
Saponin	+	++	+	+++
Flavonoids	+++	+++	+++	+++
Alkaloids	+	++	+	++
Cardiac glycosides	+	+	+	++
Terpenoids	-	+	-	+
Phlobatannins	-	+	-	+

High: +++; moderate: ++; slightly: +; absent: −.

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Table 2: Quantitative phytochemicals analysis of aqueous and ethanol okra fruits powder extracts

Okra fruit samples		Tannins (mg/100 g)	Saponin (%)	Flavonoids (mg of QE/g dw)	Alkaloids (%)	Cardiac glycosides (mg/100 g)
Control fruit powder	Aqueous Extract	90.5	4.5	13.34±2.50	3.55	50.5
	Ethanol Extract	120	9.35	15.35±3.00	8.34	95.5
IAA-treated fruit powder	Aqueous Extract	100.75	6.75	15.00±2.97	6	55.45
	Ethanol Extract	200.65	14.45	20.79*±3.65	10.59	100.55

Data are means ± SD (n = 3). * Significant differences at P < 0.05 to aqueous and ethanol extracts of the samples.

Conclusion

In this study, we conclude that the okra fruit may be used as a potential drug due to the presence of tannins, flavonoids, alkaloids, terpenoids, and saponin. These bioactive compounds play vital roles in a healthy life. The medicinal and physiological values of okra fruit are associated with these phytochemicals and nutritional constituents of the okra fruit. Moreover, the results present vital information on the biochemical basis for ethnomedicine use of okra plants.

Conflict of Interest

The authors hereby declare no conflict of interest anyway in this work.

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