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Determination of Proximate Content and Phytochemical Analysis of Some Selected Grains, Fruits and Vegetables Commercially Sold in Eke Awka Market, Awka, Anambra State, Nigeria

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ABSTRACT

Six samples from cucumber (*Cucumis sativus)*, apple (*Malus domestica*), cabbage (*Brassica oleracea*), Tigernuts (*Cyperus esculentus*), Beans (*Phaseolus vulgaris*), Green peas (*Pisum sativum*) were purchased from Eke Awka market, Awka in Anambra state, and evaluated for their proximate composition and phytochemicals content using the standard procedures as described by Association of Official Analytical Collaboration International, (1995). The study revealed that moisture content of cucumber was significantly high, followed by cabbage, apple and then tiger nuts. The outcome of Phytochemical analysis showed that Alkaloids, Flavonoid Phenol and Oxalate levels were highest in cabbage; Saponin and Tannin were highest in tiger nuts while Steroids and Phytate were highest in cucumber. The fruits and vegetables analyzed contain appreciable amounts of phytochemicals and relatively high food value thus making them valuable nutraceutical sources and very useful for nutritional and medicinal purposes

Keywords: Proximate composition, Phytochemical analysis, Grains, Fruits, Vegetables

INTRODUCTION

The nutritional value of grains, fruits, and vegetables is of paramount importance for public health, particularly in developing countries, where these food groups are integral to daily diets [1,2]. These products, commercially sold in this market, are consumed by a large population segment, making them critical to understanding food security and nutritional status in the region [3]. The analysis of the proximate content and phytochemical composition of these selected grains, fruits, and vegetables is essential to assess their nutritional quality and health benefits [4,5]. Proximate content analysis provides information on the basic nutritional components such as moisture, ash, protein, fat, fiber, and carbohydrates [6]. Phytochemicals are naturally occurring compounds found in plants that have been shown to provide numerous health benefits [7]. These compounds, such as flavonoids, alkaloids, saponins, tannins, phenolics, and terpenoids, possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The presence and concentration of these phytochemicals in food items can significantly influence their health benefits and therapeutic potential [8,9]. Phytochemical analysis, identifies the presence of bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and phenolics, which are known to have numerous health-promoting properties [10]. Together, these analyses provide a comprehensive understanding of the nutritional and therapeutic potential of the selected food items. Grains, fruits, and vegetables are fundamental components of the human diet, contributing significantly to the intake of essential nutrients [11]. Grains like rice,

maize, and millet are primary sources of carbohydrates, proteins, and certain vitamins and minerals [12]. These staple foods provide the bulk of energy requirements and play a crucial role in sustaining life and promoting growth and development. Fruits and vegetables are rich in vitamins, minerals, and natural sugars, which are vital for metabolic processes, immune function, and the maintenance of healthy skin and tissues. Fiber, a prominent composition of many fruits helps to protect against chronic diseases, aid in digestion, and promote overall well-being [13,14]. However, the nutritional value of these food items can vary significantly depending on several factors, including variety, cultivation practices, harvest time, and post-harvest handling methods [15]. Consequently, there is a need to analyze the specific proximate and phytochemical content of these grains, fruits, and vegetables to assess their quality and suitability for consumption. By determining the proximate composition of grains, fruits, and vegetables in Eke Awka Market, this study aimed to offer a clearer picture of their nutritional value, which is vital for both consumers and policymakers to make informed decisions regarding diet and food security. The phytochemical profile of the selected grains, fruits, and vegetables sold will provide valuable insights into their potential health benefits, guiding consumers in making healthier food choices and contributing to better public health outcomes.

The study has shed light on the nutritional and health-promoting properties of these essential food items and provided valuable insights that can enhance consumer awareness, improve agricultural practices, and inform policy decisions aimed at promoting better nutrition and health outcomes in the region.

METHODOLOGY

Sample collection

The samples for this investigation were purchased at the Eke Awka Market in Awka, Anambra State. To avoid spoiling, the fruits and vegetables were collected in an air-ventilated environment. The samples were authenticated by a taxonomist at the Department of separated into three groups. Pesticide residues were grouped in one fraction of the samples, trace metals in the second, and micronutrients, phytochemicals, and bioactive substances in the third.

Moisture Content

This was determined according to AOAC, (1995) [16].

Two grammes (2.0g) of each sample was weighed into an oven dried crucible and placed in the oven at 105oC for three hours, following which the dish and sample were reweighed until the weight remained consistent. It was then allowed to cool in the desiccators before being weighed again. The dish's dry weight and the sample obtained. The following formula was used to compute the percentage moisture content:

$$\text{Moisture (\%)} = \frac{Loss\ due\ to\ drying \times 100}{Weight\ of\ sample\ taken}$$

Ash content

The ash content was determined using the AOAC technique (1999) [16].

Two grammes (2.0g) oven-dried samples A-F were weighed into various porcelain crucibles that had been previously dried. The crucibles and their contents were put in a muffle furnace, and the temperature was gradually increased to 450°C. The temperature was maintained at this level for four hours. The crucibles were then placed in desiccators and allowed to cool to ambient temperature before being weighed. The following formula was used to compute the percentage loss on ignition due to weight loss during combustion:

$$\text{\% Ash (dry basis)} = \frac{W_3 - W1}{W_2 - W_1} \times 100$$

Where: W_1 is weight of crucible

W_2 is weight of crucible and sample before ashing

W_3 is weight of crucible and ash

Crude Fibre

According to the AOAC, this was measured (1995) [16].

In a 200 ml Pyrex flask, two grammes (2.0 g) of the digested samples A-F were weighed and 1.25 percent H_2SO_4 was added. A watch glass was placed over the beaker, and it was slowly cooked on a hot skillet for 30 minutes. The residue was decanted into a beaker using a sintered glass crucible. On a hot plate, 200 ml of a solution containing 1.25 g of carbonate free NaOH per 100 ml was gently heated in a beaker covered with a watch glass for 30 minutes. After removing the alkali, the sample was washed twice with 50 mL boiling water. The contents of the beaker were rinsed and dried before being burned in a sintered glass crucible. Allow it cool before weighing to

ensure a consistent weight. The fibre content is calculated by dividing the difference by the sample weight reported as percentage.

$$\%\ Crude\ Fiber = \frac{Weight\ of\ ash\ (W1 - W2)}{Weight\ of\ Sample} \times 100$$

Where W1 is Weight of sample before digestion
W 2 is Weight of sample after digestion.

Crude fat

This is done using Soxhlet Fat Extraction Method AOAC, (1995) [16]

Each sample was put into weighted filter paper and placed within a Soxhlex Extractor Column, where it was extracted for 6 hours with Petroleum Ether (40-60 0C). After carefully removing the defatted samples, the solvent was evaporated at 40°C. After that, the crude fat percentage was computed as follows:

$$\text{Fat (\%)} = \frac{W_1 - W_2 \times 100}{Weight\ of\ sample}$$

Where W1 = weight of filter paper + sample before drying
W2 =weight of filter paper + sample after drying

Crude Proteins

The protein content was calculated using the AOAC's Kjehdal technique (1995) [16].

The micro Kjeldahl technique was used to determine the crude protein content of the samples. In a 500 ml round bottom Kjeldahl flask, ten grammes (10g) of dried finely powdered material was weighed. After that, 20 ml of concentrated H_2SO_4 was added, followed by 2.0g of digesting catalyst (K_2SO_4 and H_2O combination). The flask was slowly heated until it stopped foaming. The heat was raised until the digest was colourless or light green. After cooling, the digested sample was diluted to 5ml with purified water. After that, 20 ml of the diluted digest was added to the distillation flask, followed by 25 ml of 4 M NaOH solution and distillation. The distillate was collected in a receiver with 10mL of boric acid as an indicator solution. To get the pale end-point, a 0.1 M HC1 solution was utilised. Using a conversion ratio of 6.25, the crude protein was computed by multiplying the nitrogen content by the litre value.

Crude Protein = % N ×Converting factor (6.25)

$$\%\ N = \frac{\text{Titre Value} \times \text{Molarity of Acid} \times \text{Mass of Nitrogen (N)} \times \text{Dilution Factor}}{\text{Sample Weight/Volume}}$$

Carbohydrate Determination

Carbohydrate content of the food samples were determined using differential method as reported by AOAC, (1995) [16].

Carbohydrate content of each sample was obtained by proximate analysis using the difference methods.

Available Carbohydrate (%) = 100 - (% moisture + % Ash + % Protein + % Fat)

Energy value was determined using the calculation method as stated by (Onwuka, 2005) the calorific value of a food is given by Energy value of food in KJ per 100 g = (% Available Carbohydrate x 17 + % Protein x 17 + % Fat x 37) / 100 = KJ/100 g.

Quantitative Phytochemical Screening

Flavonoid Determination

The flavonoid content was determined using the Boham and Kocipai-Abyazan techniques (1974) [17]. 10 g of fruit samples were extracted at room temperature with 100 ml of 80 percent aqueous methanol and left to stand for 5 to 10 minutes. The whole solution was filtered using Whatman filter paper No. 42. (125 mm). Before being weighed, the filtrate was transferred to a crucible and evaporated to dryness. The percentage flavonoid was calculated by subtracting the percentage flavonoid.

$$\%\ flavonoids = \frac{W_2 - W_1}{Weightofsample} x\ 100$$

Where,

W_1 = *Weight of empty crucible.*
W_2 = *Weight of crucible + residue.*

Determination of Alkaloids

In a 250ml beaker, five (5) grammes of fruit samples were inserted, and 200ml of 10% acetic acid (CH_3CO_2H) in ethanol (C_2H_5OH) was added. The mixture was covered and let to stand at room temperature for 4 hours at 25°C. The filtrate was then concentrated on a water bath until it reached a quarter of its original volume, after which it was filtered using filter paper No. 42. Drop by drop, concentrated NH_4OH was added until the precipitation was complete. The precipitate was collected on weighted filter paper and rinsed with dilute NH_4OH once the mixture had settled. The alkaloid precipitate was dried and weighed. By subtracting the proportion of alkaloid, the percentage alkaloid was computed [18]

$$\%\ Alkaloids = \frac{W_2 - W_1}{Weight\ of\ sample} x\ 100$$

Where,

W_1 = Weight of empty filter paper.
W_2 = Weight of filter paper + Alkaloid.

Determination of Total Saponins

Obadoni and Ochuko's approach was used to determine saponin [18]. A conical flask was filled with exactly 20 g of sample and 100 ml of 20 percent aqueous ethanol. The sample was heated in a water bath at 55°C for 4 hours while being constantly stirred. The residue was re-extracted with 200 mL 20 percent ethanol after the mixture was filtered. Over a water bath at about 90°C, the mixed extract was concentrated to 40 ml. The concentrate was transferred to a 250 mL separatory funnel, which was then filled with 20 mL diethyl ether and violently shaken. The aqueous layer was recovered whereas the ether layer was discarded when it had settled. With 60 mL of n-butanol, the purification procedure was repeated. 10 mL of 5% aqueous sodium chloride was used to wash the mixed n-butanol extracts twice. The leftover solution was boiled in a water bath to evaporate the solvents and dried to a consistent weight in the oven. As a percentage, the saponin content was determined.

$$\%\ Saponins = \frac{W2 - W1}{Weight\ \ of\ sample} x\ 100$$

Where, W_1= Weight of filter paper.
W_2= Weight of filter paper + residue.

Determination of Total Phenols by Spectrophotometric Method

Diethylether $(CH_3CH_2)2O$ was used to boil the fat-free sample. Five millilitres of the boiling extract were pipetted into a 50-millilitre flask, followed by ten millilitres of distilled water. 2 ml ammonium hydroxide solution and 5 ml concentrated amylalcohol $(CH_3(CH_2)_3CH_2OH$ were added after the distilled water was added. For colour development, the samples were prepared up to mark and allowed to react for 30 minutes. This was observed at a wavelength of 505 nm [19].

$$Conc.ofSample\ (mg/l) = \frac{Absorbance\ \ of\ Sample\ x\ Conc.\ of\ Sample}{Absorbance\ \ of\ Standard}$$

Determination of Total Tannin by Titration

Pearson (1974) [20] described the Follin Denis titrating technique, which was employed. 100 ml petroleum ether was added to 20 g of crushed material in a sample conical flask and covered for 24 hours. After that, the sample was filtered and set aside for 15 minutes to enable the petroleum ether to evaporate. It was then re-extracted by soaking it for 4 hours in 100 mL of 10% acetic acid in ethanol. The filtrate was then collected after the sample was filtered. To precipitate the alkaloids, 25 mL of NH_4OH was added to the filtrate. The alkaloid was cooked with an electric hot plate to eliminate any remaining NH_4OH. The remaining amount was found to be 33 milliliters. 5 ml of this was mixed with 20 ml of ethanol. It was titrated with 0.1 M NaOH until a pink end point was achieved, using phenolphthalein as an indicator. Tannin content was then calculated in % ($C_1V_1 = C_2V_2$) molarity. Where, C_1 = Conc. of tannic acid, C_2 = Conc. of base, V_1 = volume of tannic acid and V_2 = Volume of base.

Determination of Phytate

The phytate content was calculated using the Young and Greaves (1940) technique, which Lucas and Markakes followed [22]. A total of 0.2 g of material was weighed into 250 mL conical flasks. Each sample was soaked for 3 hours in 100 cc of 2 percent conc. HCL. After then, the sample was filtered. Each sample was mixed with 50 mL of each filtrate in a 250 mL beaker and 100 mL of distilled water. As an indicator, 10 mL of 0.3 percent ammonium

thiocyanate solution was added, and the solution was titrated using 0.00195 g iron per 1 mL of standard iron (III) chloride solution.

Determination of Oxalate by Titration Methods

This determination involves three major steps viz digestion, oxalate precipitation, and permanganate titration [23].

Digestion:

1) 2 g of sample was suspended in 190 ml of distilled water in a 250 ml volumetric flask.
2) 10 ml of 6M HCL was added and the suspension digested at 100°C for 1 hour.
3) Cool and then make up to 250 ml mark before filtration.

Oxalate Precipitation:

4 drops of methyl red indicator were applied to duplicate portions of 125 ml of the filtrate in beakers. After that, NH_4OH solution was added drop by drop until the
test solution changed from salmon pink to a light yellow colour (pH 4-4.5). After that, each part was heated to 90°C, cooled, and filtered to remove ferrous ion-containing precipitate. The filter was heated to 90°C again, and 10 mL of a 5% $CaCl_2$ solution was added while being continually agitated. After heating, it is cooled and kept at 25°C overnight. The solution is then centrifuged for 5 minutes at 2500 rpm. The supernatant is decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution.

Permanganate Titration:

At this point, the total filtration from the digestion of 2 g of flour had reached 300 ml. Aliquots of 125 ml filtrate were heated to near boiling and titrated against a 0.05 M standardised $KMNO_4$ solution for 30 seconds to generate a slight pink colour. The calcium oxalate content was then determined.

Determination of Steroids:

In a 100ml beaker, around 0.5g of material was weighed and extracted for 30 minutes with 20ml chloroform-methanol (2:1) In a 100ml flask, the materials were filtered and dried using Whatsman filter paper (No.1). The tests were carried out again and again until the samples were proven to be steroid-free. To achieve homogeneity, 1 mL of extract was pipetted, followed by 5 mL of alcoholic KOH, and the mixture was vigorously agitated. After cooling to room temperature, 10ml petroleum ether and 5ml distilled water were added to the mixture, which was then heated for 90 minutes in a water bath at 400°C. This combination was evaporated to dryness in a water bath. After adding 6ml of Liebermann-Burchard reagent to the residue, the absorbance was measured at 620nm. The sample was treated in the same manner as a standard steroid concentration of 0-4 mg/ml would be. To calculate the steroid percentage, the following formula was used:

$$\% \text{ Steroid} = \frac{Absorbance \times Gradient\ Factor \times Dilution\ factor}{Sample\ \ weight\ in\ \ grams \times 10000}$$

RESULTS

Proximate Composition of Selected Grains, Fruits and Vegetables

The result of proximate composition of selected grains, fruits and vegetables from Eke Awka market was shown in Fig.4.1. The result showed that the moisture content of cucumber (89.35±1.02%) was significantly high ($p<0.05$) compared to others samples analysed. This is followed by cabbage (88.43±1.02) and apple (87.56±2.10%) the least was tigernuts (8.00±0.02%). Crude fat was significantly highest in tiger nuts (43.00±0.12%) followed by cabbage(2.15±0.03%)the least was apple(0.26±0.02%).The crude oil fiber was significantly high in tiger nuts (6.00±0.02%), followed by beans (3.76±0.01%) and least in apple (0.92±0.001%).The protein content of beans (22.82±0.25%) was significantly highest at ($p<0.05$) when compared to other analyzed sample, followed by tigernuts (4.45±0.03%) and least was apple (1.02±0.00%). The ash content of beans (3.45±0.02%) and green peas (2.40±0.02%) were significantly high at $P<0.05$ when compared to other analyzed samples. Carbohydrate content of beans (58.36±1.03%) was significantly highest at ($p<0.05$) when compared to other analyzed sample, followed by tiger nuts (38.05±0.23%least was cabbage (3.76±0.03%). The differences were significantly at $p<0.05$. The energy value was significantly high in tiger nuts (557.0±2.02) at $P<0.05$ when compared to other analyzed samples followed by beans (340.20±1.05%) and least in cucumber (36.39±2.15%).

Table 1: Proximate composition of grains, fruit and vegetables sold in Eke-Awka market, Awka, Anambra State, Nigeria

Composition (%)	Cucumber	Apple	Cabbage	Green pea	Beans	Tiger nuts
Moisture	89.35±1.02^{a}	87.56±2.10^{c}	88.43±1.02^{b}	84.00±2.05^{d}	9.86±0.25^{e}	8.00±0.02^{c}
Crude fat	0.35±0.03^{e}	0.26±0.02^{c}	2.15±0.03^{b}	0.51±0.01^{d}	1.72±0.02^{c}	43.00±0.12^{a}
Crude fiber	1.85±0.02^{e}	0.92±0.01^{c}	3.08±0.01^{c}	2.50±0.02^{d}	3.76±0.01^{b}	6.00±0.02^{b}
Crude protein	1.78±0.01^{c}	1.02±0.00^{c}	1.72±0.7^{d}	6.58±0.12^{b}	22.82±0.25^{a}	4.45±0.03^{b}
Ash	1.25±0.2^{d}	0.76±0.004^{e}	0.86±0.02^{e}	2.4±0.02	3.45±0.02^{b}	0.50±0.01^{a}
Carbohydrate	4.98±0.12	9.48±0.03^{c}	3.76±0.03^{e}	4.20±0.00^{e}	58.36±1.03^{a}	38.05±0.23^{b}
Energy value	36.39±2.15	44.34±2.02^{d}	41.27±1.04^{e}	47.11±207^{c}	340.20±1.05^{b}	557.0±2.02^{a}

Phytochemical Composition of Selected Grains, Fruits and Vegetables

Phytochemical content of selected grains, fruits and vegetables from Eke Awka market were shown in Fig.4.2. The result of the study showed that Alkaloids level was significantly highest in cabbage (6.01±0.03%) when compared to other analyzed samples followed by green peas (2.50±0.01%) and least in apple (1.64±0.01%). Flavonoids levels were significantly highest in cabbage (12.08±1.02) at P<0.05 when compared to other analyzed samples followed by green peas (8.46±0.21%) and least in cucumber (1.90±0.00%). Saponin level was highest in tiger nuts (4.80±0.02%) at p<0.05 when compared to other analyzed samples, followed by green peas (3.78±0.02%) and least in cabbage (0.01±0.00%). Tannis level was significantly highest in tigernuts (7.26±0.02%) at P<0.05 when compared to other analyzed samples, followed by apple (6.22±0.00%) and least in beans (0.35±0.01%) the phenols level was significantly highest in cabbage (16.42±0.5%) at p<0.05 when compared to other analyzed samples, followed by tigernuts (9.70±0.12%) and least was beans (0.14±0.00%). Oxalates level was significantly higest in cabbage (8.00±0.02%) at p<0.05 when compared to other analyzed samples followed by cucumber (1.05±0.02%) and least were green peas and beans (0.00±0.00%) and (0.00+0.00%) respectively. Steroids level were significantly highest in cucumber (8.45±0.01%) at p<0.05 when compared to other analyzed samples, followed by tigernuts (1.50±0.01%) and least were cabbage, green peas and beans (0.00±0.00%), (0.00±0.00%) and (0.00±0.00%) respectively. Phytates level were significantly highest in cucumber (2.00±0.00%) at p<0.05 wnen compared to other analyzed samples followed by cabbage (1.61±0.01%) and least was beans (0.04±0.00%).

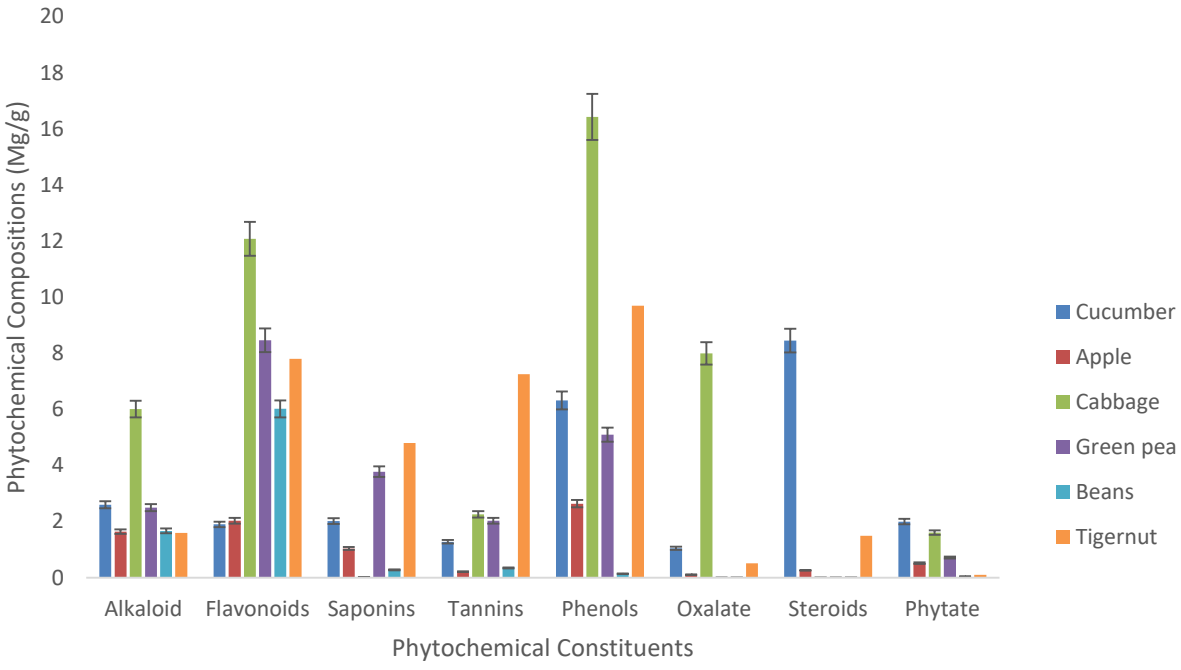

Fig 2: Phytochemical Compositions of Fruits and Vegetables from Eke Awka Market

DISCUSSION

Cucumber had the highest moisture content, followed by cabbage, apple, and green peas, according to the study. The results supported Zhang et al [24], assertion that cucumber had the greatest moisture content. Cucumber contains 96 percent water, so it may help you stay hydrated. Tiger nuts, on the other hand, had a high crude fat and fibre content, followed by cabbage. This result corroborated with Belewu's [25] findings that tiger nuts had a greater crude fat content. Increased blood cholesterol levels have been related to a high fat content in the body. As a result, it is suggested that fat be replaced with alternatives in order to lower the risk of chronic illness. The high fibre content may aid in the attraction of water to the stool, which can aid in the prevention of constipation, colon cancer, and other digestive health issues [26]. Beans have the highest protein and ash content, followed by green peas. Beans' high protein content makes them great for managing insulin resistance and diabetes. Tiger nut has the highest energy content, followed by beans. Approximately 100g of tiger nuts might supply 15-22 percent of daily energy needs for children aged 4 to 9 years, and 8-16 percent of daily energy requirements for adolescents, adults, and expectant moms. Tiger nut has a high energy level, which is in par with other nuts [27]. Alkaloids, Flavonoids, Saponin, Tannin, Phenol, Oxalate, Steroids, and Phytate are the primary phytochemicals detected in the sample examined. Several fruits and vegetables studied (e.g. cabbage and cucumber) had high alkaloid, flavonoids, phenol, and oxalate levels, while tiger nut had high saponin and tannin levels. This was in agreement with the findings of Mukherjee et al [28], on the pharmacological potential of the peels of eighteen tropical fruits. Cucumber peels include flavonoids, alkaloids, saponins, terpenoids, and steroids, according to the research. Antioxidants, cytotoxic, antidiabetic, antimicrobial, and antibacterial activity were also observed in the pharmacological research.

CONCLUSION

The fruits and vegetables analyzed contain appreciable amounts of phytochemicals and relatively high food value thus making them valuable nutraceutical sources and very useful for nutritional and medicinal purposes

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