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ETHANOLIC EXTRACT OF *CYPERUS ESCULENTUS* L. (TIGER-NUT) AN ALTERNATIVE TO FARM ANIMALS' NUTRIENT

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Abstract:

Qualitative and quantitative phytochemical screening of ethanolic extract of Cyperus esculentus L. (Tiger-nut) was carried out to determine possible medicinal and nutritive properties. Cyperus esculentus has been in use by traditional medical practitioners for treating and preventing several ailments like erectile and heart dysfunctions, diabetes etc. Results of the study revealed the presence of some bioactive compounds such as alkaloid, flavonoid, steroid, tannin, glycoside, phenol, terpenoid, and saponin. Results obtained were as follow: the percentage yield of the extract 3.83%, alkaloid (1.47 ± 0.04mg/g), flavonoid (2.55 ± 0.20mg/g), saponin (0.93 ± 0.12mg/g), tannin (0.74 ± 0.05mg/g), glycoside (0.53 ± 0.66mg/g), steroid (0.19 ± 0.12mg/g), terpenoid (0.47 ± 0.03mg/g), and phenol (0.87 ± 0.03mg/g). Results indicated that flavonoid is highly present with the highest concentration compared to other phytochemicals. The presence of these phytochemicals in tiger nuts extract could be the reason tiger nuts are used as alternative in feeding farm animals and in the management of illnesses and diseases by local herbal medical practitioners.

Keywords: Phytochemicals, Cyperus esculentus, Tiger nut, Scavengers, Antioxidants, Plant extract.

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INTRODUCTION:

Plant's medicinal values could reside in the phytochemical (bioactive) constituents of the plant with various physiological effects on human and animal health. Plant's phytochemicals may be used as base for the screening and production of modern drugs in the treatment of various diseases [1]. In Nigeria, tiger nut is known among the various tribes as: *Aki awusa* in Igbo, *Aya* in Hausa, *Ofio* in Yoruba and *Isipaccara* in Efik.

Cyperus esculentus or Tiger nut, is an edible perennial grass-like C_4 plant of the sedge family (Cyperaceae) [2]. It is rich in monounsaturated oils with phospholipids and other bioactive compounds such as tocopherols, phytosterols and polyphenols [3]. Although tiger nut oil fatty acid profile is similar to olive oil, nut oil has unique gold-yellow colour, neutral taste properties, high in phytosterol [3], and better deep-frying stability [4].

The nutritional profiles and unique functional properties have made tiger nut as unique food [5] like beverage, flour [6], edible oil [7], and a feed source [8].

Tiger nut milk has been tried as an alternative source of milk in fermented products [9] such as yoghurt production and other fermented products common in some African countries and can thus be useful replacing milk in the diet of people intolerant to lactose to a certain extent [10, 11, 12]

In ayurvedic medicine tiger nuts are used in the treatment of flatulence, diarrhoea, dysentery, debility and indigestion [13, 14, 15, 16]. Tiger nut oil has been in use by locals, in the cosmetic industry, as an antioxidant due to its vitamin E content. Unpublished data and inquiries from local cosmetologists in southeast Nigeria claim that tiger nut oil slows down ageing of the body cells, enhances skin texture and elasticity by reducing formation of skin wrinkles.

Aim and Objectives

The study objective was to study phytonutrients present in the edible *Cyperus esculentus*. Study was aimed at providing healthy nutritional alternatives for feeding farm animals and poultry. This would serve as better economic alternatives to agropreneurs for maximum profits in their business.

MATERIALS AND METHODS:

Sample Collection

Cyperus esculentus L. used for this study were purchased from a popular market in Enugu State, Nigeria. Samples were properly identified and authenticated by Prof. Eze, C. U. of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology (ESUT), Nigeria.

Reagent Preparation

Wagner's reagent

Iodine crystals, 13g and 2.0g of potassium iodide were weighed using electric weighing machine and dissolved in 80ml of distilled water and made up to 100ml in a 100ml volumetric flask.

Fehling solution A

$CuSO_4.5H_2O$ (34.66g) was weighed and dissolved in a volumetric flask with distilled water and diluted up to 500ml.

Fehling solution B

Potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) (173g) and 50gof NaOH was weighed and dissolved in distilled water and it was diluted to 500ml when cold.

85% phosphoric acid

Phosphoric acid (85ml) was measured with a measuring cylinder and poured into a 100ml volumetric flask and made up to mark with distilled water.

Hager's reagent

Picric acid (1g) in 100ml of water

10% Ferric chloride solution

Ferric chloride (10g) was weighed using electric weighing machine into a 100ml volumetric flask and made up to 100ml with distilled water.

20% sodium carbonate (IV)

Sodium carbonate (IV) (20g) was weighed into a 100ml volumetric flask and made up to100ml with distilled water.

10% acetic acid in ethanol

Acetic acid (10ml) was measured using measuring cylinder and poured into a 100ml volumetric flask made up to mark with ethanol.

80% ethanol

Ethanol (80ml) was measured using measuring cylinder into a 100ml volumetric flask and made up to the mark with distilled water.

1% Ferric chloride solution

Ferric chloride (1g) was weighed using electric weighing machine into a 100ml volumetric flask and made to the mark with distilled water.

1% HCl
Hydrochloric acid (1ml) was measured using measuring cylinder into a ml volumetric flask and made up to the mark with distilled water

10% sodium hydroxide
Sodium hydroxide pellets (10g) were weighed using electric weighing machine into a 100ml volumetric flask and made up to the mark with distilled water.

60% Sulphuric acid
A volume (60ml) of sulphuric acid was measured into a 100ml volumetric flask and made up to the mark with distilled water.

15% lead acetate
Lead acetate (15g) was weighed using electric weighing machine and was dissolved with distilled water and made up to mark in a 100ml volumetric flask with distilled water.

20% Sulphuric acid in ethanol
Sulphuric acid (20ml) was measured using measuring cylinder into a 100ml volumetric flask and made up to the mark with ethanol.

5% phosphomolybdic acid
Phosphomolybdic acid (5g) was weighed using electric weighing machine and was dissolved with distilled water and made up to the mark in a 100ml volumetric flask.

Dragendorf's reagent
Bismuth nitrate (0.5g) was weighed into an empty beaker and 10ml of distilled water was added to it. 10ml of conc. HCL was added to the mixture. 4g of potassium iodide was weighed into another beaker and was dissolved with small quantity of distilled water till the potassium iodide is completely dissolved. The two solutions from different beakers were mixed.

5% ferric chloride
Ferric chloride (5g) was weighed using electric weighing machine and was dissolved in a 100ml volumetric flask with distilled water.

0.1N Hydrogen chloride acid (HCl)
Hydrochloric acid (8.177ml of 12.23M (37%) was diluted to 1 litre with distilled water.

0.008M potassium ferricyanide
Potassium ferricyanide $K_3FeC(N)_6$ (3g) was weighed into a 1000ml volumetric flask and dissolved with distilled water (1litre).

Folin-Ciocalteu's reagent
Sodium tungstate (10g) and 25g of sodium molybdate was weighed and dissolved with 70ml of distilled water in a volumetric flask. 5ml of 85% phosphoric acid and 10ml concentrated hydrochloric acid were measured and added to it and the mixture was refluxed for 10hours. 15g of lithium sulphate was added together with 5ml of water and 1drop of bromine. Later, it was refluxed and brought to 100ml with distilled water.

SAMPLE PREPARATION/EXTRACTION
A quantity (300g) of sample were washed, dried and later weighed using digital weighing balance. Weighed samples were milled to fine particles which were immediately transferred into transparent container and soaked in 800ml of ethanol and left for 24hours. Soaked mixture were extracted in Soxhlet apparatus connected to a rotatory evaporator at 55˚C to concentrate the crude extract. The concentrated crude extract was used for the analysis.

SAMPLE ANALYSIS
Qualitative analysis Qualitative phytochemical screening of the ethanolic extract of tiger nut was carried out to determine the presence of the following phytochemicals: alkaloid, flavonoid, saponin, phenol, glycoside, terpenoid and tannin as described by Harborne (1973) [10]

Test for alkaloid
The extract (0.1g) was weighed into a separate test tube and 1ml of 1% HCL was added to each, heated in a water bath for 20minutes, allowed to cool and 1ml of the heated extract was pipetted into a separate test tube and 0.5ml of Mayer's reagent was added to each. Expected colour was creamy colour which shows the presence of alkaloid.

Test for flavonoid
The extract (0.1g) was weighed into test tubes and 10ml of distilled water was added to each. The solution was shaken and 1ml of 10% NaOH was added to each. A yellow colour indicates the presence of flavonoid.

Test for saponin
The extract (0.1g) was boiled with 5ml of distilled water and filtered. To the filtrate about 3ml of distilled water was further added and shaken vigorously. Frothing which persisted on warming was taken as an evidence for the presence of saponin.

Test for steroid
The extract (0.1g) was pipetted into test tube and 5

drops of sulphuric acid was added to it. Colour changes to red show the presence of steroids.

Test for tannin
The extract (0.1g) was weighed and stirred with 10ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of the filtrate. The presence of a blue black or blue green precipitate indicated the presence of tannin.

Test for glycoside
The extract (0.1g) was mixed with 300ml of distilled water and heated on a water bath for 5 minutes and filtered. To 5ml of the filtrate, 0.2ml of Fehling solution A and B until it turns alkaline. The solution was heated on a water bath for 2minutes. A brick red precipitate indicates the presence of glycoside.

Test for terpenoid
The extract (0.1g) was dissolved in ethanol acetic anhydride (1ml) was added, followed by the addition of conc. Sulphuric acid. A change in colour from pink to violet showed the presence of terpenoid.

Test for phenol
The extract (0.1g) was boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution were added. A green blue or violet colouration indicates the presence of a phenolic dihydroxyl group.

Quantitative Analysis
Quantitative phytochemical analysis was carried out on the ethanolic extract of tiger nut (*Cyperus esculentus* L.) to determine the absolute quantity of the parameters present in sample following the method described by Harborne (1973) [10]

Quantification of alkaloid content
A quantity (0.1g) of the extract was weighed out separately, mixed and shaken with 20ml of 20% sulphuric acid in ethanol (1:1) and was filtered. The filtrate (1ml) was pipetted out and 5ml of 60% sulphuric acid was added. After 5minutes, 5ml of 0.5% formaldehyde in 60% sulphuric acid was also added. The mixture was allowed to stand for 3hours and absorbance at 550nm was read.

Quantification of flavonoid content
A quantity (0.1g) of the extract was weighed out separately, macerated with 20ml of ethyl acetate, filtered through Whatman No.1 filter paper and 5ml of the filtrate was pipetted out. Dilute ammonia (5ml) was added, shaken and the upper layer of the mixture was collected. Absorbance at 490nm was measured.

Quantification of saponin content
A quantity (0.1g) of the extract was weighed was weighed and macerated with 20ml of petroleum ether, decanted into a beaker and washed again with 10ml of petroleum ether. The filtrates were combined and heated to dryness. The residue was dissolved with 6ml of ethanol. The 2ml of the dissolved residue was put into a test tube and 2ml of chromogen solution (4ml iron stock made up to 24ml with concentrated sulphuric acid) added to it. It was allowed to stand for 30minutes and the absorbance was read at 550nm against an ethanol blank.

Quantification of steroid content
A quantity (0.1g) of the extract was weighed out separately, macerated with 20ml of ethanol and filtered through Whatman No.1 filter paper. The filtrate 2ml was pipetted out and 2ml of chromogen solution (4ml iron stock made up of 24ml with conc. sulphuric acid) was added and the mixture was left to stand for 30minutes. Absorbance was measured at 550nm.

Quantification of tannin content
A quantity (0.1g) of the extract was weighed; 50ml of distilled water was added and filtered. Then 5ml of the filtrate was pipetted out into test tube and mixed with 0.1N ferric chloride in 0.1N HCl and 0.008N potassium ferricyanide. The absorbance was measured at 720nm.

Quantification of glycoside content
A quantity (0.1g) of the extract was weighed out separately, macerated with 20ml of distilled water and 2.5ml of 15% lead acetate was added to the filtrate, shakes vigorously and the lower layer collected and evaporated to dryness. Glacial acetic acid (3ml) was also added together with 0.1ml of 5% ferric chloride and 0.25ml of concentrated sulphuric acid. The mixture was shaken and put in a dark for 2hours. Absorbance was measured at 530nm.

Quantification of terpenoid content.
A quantity (0.1g) of the extract was weighed out, macerated with 20ml of ethanol and weighed out, macerated with 20ml of ethanol and filtered through Whatman No. 1 filter paper. The filtrate (1ml) was pipetted out and 1ml of 5% phosphomolybdic acid solution was added and shaken. Gradually 1ml of concentrated sulphuric acid was added to the solution. The mixture was left to stand for 30minutes. Ethanol (3ml) was added and absorbance was measured at 700nm.

Quantification of phenol content
A quantity (0.1g) of the extract was weighed out,

macerated with 20ml of 80% ethanol and filtered through Whatman No.1 filter paper. The filtrate (5ml) was pipetted out and 0.5ml of Folin-Ciocalteu's reagent was added. After 30 minutes, 2ml of 20% sodium carbonate (IV) was added and the mixture was left to stand for 10minutes. Absorbance was measured at 650nm.

RESULTS:

Table 1: RESULT OF EXTRACTION/YIELD

Solvent	Weight of Sample (g)	Weight of Extract (g)	Yield (g)	Yield (%)
Ethanol	300	11.50	0.0383	3.38

Table 2: Results of the qualitative phytochemical analysis of the ethanolic extract of *Cyperus esculentus* L. (Tiger nut)

Phytoconstituents	Abundance
Alkaloid	++
Flavonoid	+++
Saponin	++
Steroid	+
Tannin	++
Glycoside	+
Terpenoid	++
Phenol	++

The above table is a summary of the qualitative phytochemical analysis of the ethanolic extract of *Cyperus esculentus* L. (tiger nut).

LEGEND
+++ = Highly present
++ = Moderately present
+ = Slightly present

Table 3: Results of the quantitative phytochemical analysis showing the concentration of each phytochemical constituent present in the ethanolic extract of *Cyperus esculentus* L. (Tiger nut).

Parameter	Concentration (mg/g) Mean ±SEM
Alkaloid	1.47 ± 0.04
Flavonoid	2.55 ± 0.20
Saponin	0.93 ± 0.12
Steroid	0.19 ± 0.12
Tannin	0.74 ± 0.35
Glycoside	0.53 ± 0.66
Terpenoid	0.47 ± 0.03
Phenol	0.87 ± 0.03

Results are presented as mean ± standard deviation, n=3.

DISCUSSION:

Tables 1, 2, and 3 show the results of the extract yield, the qualitative and quantitative phytochemical analyses of the ethanolic extract of *Cyperus esculentus* L. (Tiger nut)

While result in Table 1 shows the percentage yield of the extract, Table 2, revealed the presence of several bioactive compounds like steroid, flavonoid, saponin, alkaloid, terpenoid, tannin, glycoside, and phenol. It revealed that in ethanolic extract of tiger nut *(Cyperus esculentus* L.*)*, the concentration of flavonoid is higher (+++) when compared with glycoside (+).

Table 3 shows the concentration, in mg/g, of the aforementioned phytochemical constituents and revealed that the flavonoid (2.55±0.20mg/g) has the highest concentration, compared to alkaloid (1.47±0.04mg/g), saponin with (0.93±0.12mg/g), and tannin having this (0.74±0.05mg/g). Meanwhile, glycoside with (0.53±0.66mg/g) is relatively higher in concentration than terpenoid (0.47±0.03mg/g). Also, phenol (0.87±0.03mg/g) is higher in concentration than glycoside while the plant phytochemical: steroid has the lowest concentration (0.19±0.12mg/g) among all phytochemical assayed.

Elsewhere, scientific evidence has shown that these phytochemicals: flavonoid, alkaloid, steroid, saponin and tannin posses antimicrobial properties [12]. Alkaloids inhibit some mammalian enzyme activities and also affect glucagon and thyroid stimulating hormones. This function makes them to be used as analgesic, antispasmodic and antibacterial agents [14]. Saponin have been reported to be useful in reducing inflammation of the upper respiratory passage and also chiefly as foaming and emulsifying agents and detergent [12]. This study shows the potential of tiger nut oil or its extracts in this utilisation. Again, this could further be exploited as nutritional components at least in farm animals as we advocate. Tannin with its astringency hastens wound healing and aids in the prevention of tooth decay [8]. Tannin compounds have antimicrobial properties which make them useful in the prevention and treatment of urinary tract infection and other bacterial infection [10]. These infections, which are also common in humans can be easily treated, naturally, following proper utilisation of this extract. This extract could be used in animal husbandry for chemotherapeutic purposes thereby reducing the over-dependence on antibiotics in rearing of domestic animals and birds for food. Reduction in the use of chemicals in rearing domestic animals also improves human health thus reducing various diseases that could be associated with eating unsupervised animals and birds slaughtered for meat purposes.

Flavonoids are known to have antioxidant effects and could hinder the initiation and progression of tumours while reducing the progression of coronary heart disease [11].

Alkaloids inhibit certain mammalian enzyme activities and also affect glucagon and thyroid stimulating hormones; some have been used as analgesic, anti-spasmodic and anti-bacterial agents. Flavonoids are known to have antioxidant effects and have been shown to initiation, promotion and progression of tumours; reduce coronary heart diseases. Tannin has astringent properties that hastens wound healing. It aids in the prevention of tooth decay and also responsible for preventing and treating urinary tract infection and other bacterial infections. Saponin is useful in reducing inflammation of the upper respiratory passage while glycosides have therapeutic benefits as they possess expectorant properties [11]. Steroid has anti-inflammatory agents, with growth-stimulating agents and oral contraceptive properties. Terpenoids have the ability to reduce heart burn and gastric acid reflux.

Phenols, which are strong antioxidants with potential of chelating metal ions involved in production of free radicals, is present in this extract. This presence could be exploited in formulating this as feed and drug for animal husbandry. This will reduce the overdependence on use of synthetic chemicals in rearing of animals and birds.

CONCLUSION:

Medicines derived from plants have made immense contribution towards the betterment of human health and act as a source of inspiration for novel drug compounds.

This study has shown that ethanol extract of *Cyperus esculentus* L. has immense potential as natural drug and food substance in drug and nutrient formulation for feeding animals and birds.

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