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Comparative Phytochemical Screening and Antioxidant Activity of Ethanol Extracts of Fresh and Dried Cyperus Esculentus

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ABSTRACT
Phytochemicals are bioactive molecules present in plants. Some of them have antioxidant activities that impact on human health. This study assessed the phytochemical composition and antioxidant activities of ethanol extracts of fresh and dried Cyperus esculentus nut by standard methods. Both fresh and dried Cyperus esculentus nut contained saponin, flavonoid, phenol, steroids, terpenoid, terteoids, anthroquinone, cyanogenic and cardiac glycosides, tannins, alkaloids and reducing sugar. Quantitatively, dried Cyperus esculentus nut had higher concentrations of these phytochemicals than the fresh nut. Fresh nut had alkaloid content of 1.8%, flavonoid 12.2%, tannins 0.63mg/l, phenol 1.0% and glycosides 0.75 % while the dried nut had alkaloid content of 4.0%, flavonoid 32.0%, tannins 2.63mg/l, phenol 1.4% and glycosides 0.54%. The antioxidant capacity in fresh nuts using phosphomolybdenum, hydrogen peroxide and reducing power assays where 102ug/ml, 65.90ug/ml and 154.0ug/ml respectively while the dried extract had 166.17ug/ml, 34.0ug/ml and 332.0mglml. Cyperus esculentus is an under-utilized crop with enormous potentials. It should be part of dietary consumptions because of its phytochemical contents and antioxidant benefits.
Key words: Cyperus esculentus, fresh, dried, composition, comparism.

INTRODUCTION
Cyperus esculentus (tiger nut) belongs to the family of cyperaceae which are monocotyledonous plants with many species. Cyprus esculentus is the edible specie variously called earth almond, zulu nut, yellow nut sedge, rush nut, duck potato, edible rush or yellow nut grass . It is a root crop which grows widely in wet places as a grass and sometimes cultivated for its small sweet tubers. Cultivation time is April to November (Simpson and Inglis, 2007). Tiger nut is not actually a nut but a small tuber which appears long or round in shape with a dimension of 8mm to 16mm and can be eaten raw, roasted, dried, baked or made into a refreshing beverage (Oladele and Aina, 2007). The leaves sprout in ranks of three from the base of the plant and about 5-20cm long .The flower stems have a triangular cross section, the flower is bisexual and has a three stigma carpel with flower head having 3-8 unequal rays (Kuner et al., 2002). Phytochemicals are non-nutritive, natural and biological active chemical compounds found in plants. Phytochemical, from the Greek word 'Phyto', means plant or Phyto - constituents responsible for protecting plants from infection, damages or predators. They contribute to plant colour, aroma, flavour and have been catalogued by their protective function, physical and chemical characteristics (Meagher and Thompson, 1999). An antioxidant prevents delays or removes oxidative damage to a target molecule (Hallwell and Gutteride, 1999). Body enzymes, exposure to environmental pollution, ultraviolet light, and cigarette smoke, excessive intake of iron and food additives can convert oxygen to hydroxyl radicals. These generated radicals are highly reactive and able to cause damage to the body cell proteins, deoxyribonucleic acid and fats. The damage contributes to the emergence of degenerative diseases including coronary heart, inflammatory disease, cancer and reperfusion injury (Dai and Mumper, 2010). Oxidation

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reactions produce free radicals that initiate multiple chain reactions that cause damage or death to cells. Antioxidants remove these free radicals intermediates by being oxidized themselves and stopping the harmful chain reactions (Meagher and Thompson, 2011).

Rationale of Study

When produced in excess free radicals and oxidants give rise to oxidative stress, a harmful process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins and deoxyribonucleic acid (Genestra, 2007). Oxidative stress arises when cells cannot adequately destroy the excess free radicals formed. This simply means that oxidative stress results from an imbalance between formation and neutralization of reactive oxygen species and reactive nitrogen species and if not controlled, can induce a variety of chronic and degenerative diseases as well as the ageing process and some other acute pathology. Cyperus esculentus is a cheap, common and under-utilized crop in Africa, including Nigeria. Most people consume its nuts fresh or dried and unaware of the constituents, nutritional value, health benefits and health implications of the form in which it is consumed. It is therefore the aim of this study to compare qualitatively and quantitatively the phytochemical composition and antioxidant properties of fresh and dried Cyperus esculentus nuts.

Fig: 1 Diagram of *Cyperus Esculentus* Plant and Tubers

MATERIALS AND METHODS

Preparation of Plant Extracts

Cyperus esculentus nuts were washed with tap water and divided into two parts, one part was sun dried while the second part was fresh. The sun dried and fresh nuts were separately grounded to fine powder with electronic blender. The pulverized samples were kept in

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cellophane bags at ambient temperature. Two hundred grams of the pulverized fresh Cyperus esculentus nuts were placed in a round bottom flask containing 250ml of ethanol. The dried sample was also treated like the fresh sample. The different flasks were agitated and allowed to stand for 24 hours. The mixtures were then filtered separately using filter papers and concentrated in a water bath.

Qualitative Phytochemical Screening

Qualitative Phytochemical screening was carried out in both dried and fresh extracts to determine the presence of the following secondary metabolites, alkaloids, tannins, flavonoid, saponins, phenols, terpenoids, cynanogenic glycosides, cardiac glycosides, anthaquinone, sterioids, reducing sugar and terteoids using the methods of Sofowora (1993), Evans and Trease(1999 & 2002).

Quantitative Phytochemical Analyses

Flavonoids, alkaloids, phenols, tannins and glycoside were quantitatively assayed in both extracts. Flavonoid was determined by the method of Bohn and Kopcipal, alkaloid by the method of Harborne while others were assayed spectrophotometrically.

Antioxidants Analyses

Antioxidants analyses were carried out using both extracts of fresh and dried tiger nuts. Reducing power was determined by the method of Oyaizu, phosphor- molybdenum by the method of Prieto et al. and hydrogen dioxide with the method of Ruch et al.

RESULTS

Table 1: Qualitative Phytochemical Analyses of Fresh and Dried Cyperus Esculentus

S/N	PARAMETERS	FRESH NUT	DRIED NUT
1	Saponin	++	++
2	Flavonoids	+	+++
3	Phenols	++	+++
4	Steroids	++	+
5	Terpenoid	++	+++
6	Anthraquinone	+	++
7	Terteoids	++	+++
8	Cyanogenic glycosides	++	++
9	Cardiac glycosides	++	++
10	Tannins	++	+++
11	Alkaloids	++	+++
12	Reducing Sugar	+++	+

KEY

+++ = Present in high abundance
++ = Moderately present
+ = trace amount

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Table 2: Quantitative Phytochemical Composition of Fresh and Dried Cyperus Esculentus Nut

Cyperus esculentus	glycosides %	Tannins Mg/100g	Alkaloid %	Flavonoid %	Phenol %
Fresh	0.75	0.63	1.8	12.2	1.0
Dried	0.54	2.63	4.0	32.0	1.4

Table 3: Antioxidant Capacity of Fresh and Dried Cyperus Esculentus Nut

S/N	Parameters	Fresh (ug/ml)	Dried (ug/ml)
1	Reducing power	154.0	332.0
2	Phosphomolybdenum	102.35	166.17
3	Hydrogen peroxide	65.94	34.0

DISCUSSION

Phytochemicals are large group of plant derived - compounds responsible for much of the disease protection conferred from diet high in fruits, vegetables, beans, cereals and plant based beverages such as tea and wine (Karrie et., 2008).Other sources include legumes, nuts, seeds, fungi, herb and spices (Mathai, 2008). Broccoli, cabbage, carrot, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, rash berries and soya food are also common sources. Some phytochemical molecules are endowed with antioxidant properties that protect cells from oxidative damage and reduce the risk of developing terminal illnesses.

This study presents systematic qualitative and quantitative analyses of phytochemical constituents and antioxidant capacities of fresh and dried nuts of Cyperus escnlentus (tiger nut). Qualitative analysis indicated the presence of different secondary metabolites such as alkaloids, flavonoid, glycosides, anthraquinones, phenols, terteoids, tannins, saponins and reducing sugar in both fresh and dried nut and suggests that both fresh and dried Cyperus esculentus have medicinal potentials and can be therapeutically utilized especially with the dried nuts. Quantitatively, most of the secondary metabolites were present in higher concentration in the dried extract than in the fresh tiger nut (Tables1&2). This is due to the removal of water molecule and concentration of substances. Flavonoids occurred more in dried (32%) than in fresh nut (12.2%). Flavonoids are polyphenolic compounds that are ubiquitous in nature and occur as aglycones, glycosides and methylated derivatives. They have multiple biological and pharmacological activities and possess the capacity to act as powerful antioxidant to protect the body from free radicals and reactive oxygen species. The ability to behave as antioxidant depends on the molecular structure. The position of the hydroxyl group and other features in the chemical structure of flavonoids is important to their antioxidant and the free radical scavenging activities. Flavoniods constituent a wide range of substances that play important role in protecting biological systems against the harmful effect of oxidative process on macromolecules such as carbohydrate, protein, lipids and deoxyribonucleic acid (Atman et al., 2009). Dried tiger nut is a better provider of tannins (2.63 mg/100g) than the fresh nut (0.63 mg/100g). Tannins are heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with protein, alkaloids, nucleic acids and minerals (Schofields et al., 2001). Tannins convert materials into leather (tan) and are soluble in water and alcohol. Tannins help to heal burns, improve digestion, stop bleeding, prevent infection and possess

anti-inflammatory effect which makes it good for controlling gastritis and irritating bowl disorder. They act as astringents against diarrhea, as diuretic against stomach and duodenal tumours (De Brughe et al., 1999). Other applications are in dye stuff industry as caustics for cationic dyes (tannin dye), in the production of inks (iron gaute ink), in textile dye and as coagulant in rubber production. Tannins are used to clarify beer, wine and fruit juices in food industry (Firn, 2010). The concentration of alkaloid in dried extract was 4.0% but 1.8% in fresh nuts. Alkaloids are natural products with heterocyclic nitrogen atoms, are basic in character and possess bitter taste (Mishra, 1989). The different classes of alkaloids based on the heterocyclic ring system include pyrolidine alkaloids, pyridine alkaloids, pyrrolidine - pyridine alkaloids, pyridine – piperidine alkaloids, quinoline alkaloids and isoquinoline alkaloids. Alkaloids possess antibacterial and antifungal activities and ensure the protection and survival of plants against microorganisms. Pharmacologically they are used as anti-hypertensive and antimalaria (quinine). Some alkaloids have stimulant property as caffeine and nicotine (Adejuyitan, 2011). Fresh tiger nut contains more glycosides (0.75%) than the dried nut (0.54%) but generally in low concentration. Glycosides are condensation products of sugars with a lot of different varieties of organic hydroxyl compounds arranged in such a way that the hemactin entity of the carbohydrate essentially takes part in the condensation (James, 2013). Glycosides have a role of defense against invasion of tissue by microorganisms subsequent to wounding since many aglycones are aseptic and hence bactericidal in character.

Glycosides of medical plant can be used as cardiac stimulant, sinosides or local irritants and against capillary fragility (William, 1997). Phenols occurred more in dried than in the fresh nut. Phenolics are the hydroxyl group (-OH) containing class of chemical compounds where the group (-OH) bonded directly to an aromatic hydrocarbon group (Walton et al., 2003). Phenolics increase bile secretion, reduce blood cholesterol and employed in the treatment of skin infection. Saponin content was the same in both fresh and dried nuts while steroids and reducing sugar were more abundant in fresh nut. Saponins form stable foam in aqueous solution and consequently employed in the manufacture of extinguisher foam, tooth paste, shampoo, liquid soap and cosmetics. Plant steroids also called cardiac glycosides are therapeutically used as arrow poison or cardiac drugs. Anabolic steroids promote nitrogen retention in osteoporosis and in animals with wasting illness (Madziga et al., 2010). Terpenoids were detected more in dried nuts. They are a class of natural product derived from five carbon isoprene units. Most terpenoids have multi cyclic structures that differ from one another by their functional group and basic carbon skeleton (Elbein and molynelix, 1999). Commercially terpenoids are of use as flavour of fruits and flagrance of flowers in food and cosmetics and also important for the quality of agricultural products. Antioxidants inhibit the oxidation of other molecules. Enzymatic antioxidants include a network of detoxifying enzymes such as superoxide dismutase, peroxidase and the catalases. The glutathione system involves glutathione s-transferase. This system is present in animals, plants and microorganisms. These enzymes are at high concentration in the liver and serve in the detoxification metabolism (Hayes et al., 2010). Non-enzymatic antioxidants include vitamin C (ascorbic acid), glutathione, melatonin, tocopherols (vitamin E) and uric acid. The results of the antioxidant capacity showed that Cyperus

esculentus nut has antioxidant capacity that is highly beneficial to human health. Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity (kalcher et al., 2009). Phenols and polyphenols are the target analytes in many such cases, they may be detected by enzymes like tyrosinase or other phenol oxidases or by plant tissues containing these enzymes (Pellegrini et al., 2009). There are numerous antioxidants in dietary plants such as carotenoids, plenolic compounds, flavonoids, proanthocyanidins and benzoic acid derivatives .The cumulative and synergistic activities of the bioactive molecules present in plant food are responsible for their enhanced antioxidant properties. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor synergist and metal chelating agent, co –antioxidants or gene expression regulator to detoxify reactive oxygen specie by two major mechanisms. One mechanism involves a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the system.

The other process is the removal of reactive oxygen species /reactive nitrogen specie initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants act in the defense system at different levels such as preventive, radical scavenging, repair and de novo and adaptation where the signal for the production and reactions of free radicals induce formation and transport of the appropriate antioxidant to the right site. This paper has thrown much light on the constituents and antioxidant capacity of fresh and dried Cyperus esculentus nuts. Among food components fighting chronic diseases, great attention should be given to phytochemicals which are plant derived molecules endowed with steady antioxidant capacity. It will be highly healthy to incorporate tiger nut in diets to help the human body to mop-up free radicals, reduce oxidation, relieve oxidative stress and lower the incidence of cardiovascular and other age – related disorders. It is recommended that the Government should encourage farmers towards large-scale cultivation of Cyperus esculentus and to introduce bakers to the use of tiger nut flour to reduce over dependence on wheat flour. Pharmaceutical companies are advised to employ tiger nut constituents in the production of drugs. There is need for the establishment of small scale industries for the production of tiger nut oil, cosmetics, paper making and mat production to boast national economy and reduce unengaged manpower in the society.

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