

DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS, ACUTE TOXICITY AND HEAVY METALS PROFILE OF *Eleusine coracana* SEEDS

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ABSTRACT

Medicinal plants have been used for a very long time in the prevention and treatment of many ailments plaguing mankind. They contain phytochemicals and minerals which contribute to the pharmacological activities they exhibit. This study was carried out to investigate the proximate analysis, phytochemical and mineral constituents of the powdered seed of Eleusine coracana as well as to determine the acute toxicity profile of the powdered seeds. The results obtained showed that the seeds have low percentage moisture content $0.38 \pm 0.14\%$ (which is beneficial for longer shelf life); Total Ash value $2.23 \pm 0.13\%$, Acid Insoluble Ash value 0.76 ± 0.05). The phytochemical constituents present are alkaloids, saponins, terpenoids, flavonoids, carbohydrates, proteins, phenols, alkaloids and reducing sugars. The heavy metals and other mineral analysis showed that heavy metals such as; cadmium (Cd) and nickel (Ni) were absent while lead was detected in a negligible concentration of 0.01 ± 0.00 ppm. The essential and non-essential minerals contents of the sample detected (calcium, iron, potassium, sodium, magnesium, copper, zinc and chromium) were within the WHO specified limits. The acute toxicity profile showed that at a dose of 5000 mg/kg body weight the crude extract of Eleusine coracana did not produce any toxic effect and no mortality was observed even after 14 days of observation. The rich properties and phytoconstituents of Eleusine coracana seeds could be responsible for its nutritional status. The seeds can further be explored for pharmaceutical leads through bioassay-guided isolation and characterization of the active principles and for use as nutraceutical supplements.

Keywords: Antioxidants, Phytoconstituents, *Eleusinecoracana*, Ash values, free radicals.

INTRODUCTION

For a very long time, plants have been used by different people in different parts of the world for the prevention and treatment of various diseases or ailments, and it is currently estimated that about 80 % of the world's population relies on plants for managing disease conditions. Monierand El-Ghani (2016), and Sandhya *et al.*, (2016). Plants secondary metabolites are

expressed in different parts of plants (leaves, flowers, roots, fruits, and stems) and have been shown to exert different pharmacological activities which have been harnessed by man to treat different diseases or used as starting materials to synthesize new drugs. Kabera *et al.*, (2014), and Sofowora *et al.*, (2013). Medicinal plant products (herbal and dietary supplements, decoctions, concoctions, tablets, and capsules) are currently being used both in

the developed and developing countries of the world for the management of diseases, weight reduction and as cosmetics. Examples of such plants are; *Polyalthia longifolia* used in the treatment of gout, Singh *et al.*, (2010), *Eleusine coracana* for the treatment of diabetes and weight management, Oseghale *et al.*, (2016), *Zingiber officinale* for the treatment of malaria, Edeoga *et al.*, (2005), *Ocimum gratissimum* for the treatment of pile, Borokini *et al.*, (2013), *Vernoniacinerea* for the treatment of kidney and urinary bladder stones Thamizhmozhi *et al.*, (2015) and *Marrubium vulgare* for the treatment of liver disorder Mohammad *et al.*, (2016).

According to Mahomoodally, (2013), plant phytoconstituents produce their pharmacological effects by working synergistically, additively and sometimes individually to improve human health. In most cases, the synergistic and or additive effects are more pronounced than the individual constituents Mahomoodally, (2013).

Plants secondary metabolites are intermediate products obtained from normal metabolic processes that occur within the plant, which are not necessary for major processes in the plant such as growth, reproduction and general development Joash and Yau, (2014) and Ruby and Rana (2015). They are however used by plants against predators, environmental threats and for the expression of color pigments in the plants (Joash and Yau, 2014). They can be classified into five major groups which are; phenols, alkaloids, volatile oils, glycosides and terpenoids as the major metabolites, Singh, (2016). Phenols are the most common secondary metabolites found in plants with a chemical structure of an

aromatic ring with an attached hydroxyl group. Flavonoids, anthocyanins and phenolic acids belong to the class of phenols. They are known for their powerful antioxidant activity thereby aiding in the prevention and management of diseases associated with oxidative stress such as cancer, diabetes, atherosclerosis, neurodegenerative diseases and diseases that affect the central nervous system Obrenovich *et al.*, (2011). Alkaloids are secondary metabolites with a low molecular weight having a characteristic heterocyclic ring with nitrogen atom embedded giving them an alkaline nature. They are known to play a significant role in the protection of plants from pathogens and predators. They are known to be of therapeutic benefit to man e.g. quinine, used in the treatment of malaria, while others can be toxic e.g. nicotine Helio and Arthur, (2015). Volatile oils sometimes referred to as essential oils in plants are aromatic natural oils that are utilized for their antiviral, antibacterial and antifungal properties Kabera, (2014). Glycosides are important secondary metabolites in a plant that have a unique structure comprising the glycone part (sugar moiety) and the aglycone part joined together by a glycosidic bond which could be oxygen, sulphur, carbon or nitrogen glycosidic bond depending on the nature of the glycoside. Different types of glycosides exist such as; the cardiac glycosides, anthraquinone glycosides, phenolic glycosides, coumaric glycosides, thioglycosides, saponin glycosides and chromone glycosides Bartnik and Facey, (2017). Glycosides are known for their different pharmacological activities depending on the type of glycoside (for example; anthraquinone glycosides have laxative properties and are also being used

in the treatment of psoriasis Bartnikand Facey, (2017), cardiac glycosides are used in the management of circulatory disorders Swati *et al.*, (2015), coumaric glycosides are used as blood thinners as well as a smooth muscle relaxant Vaidehi and Rajesh, (2016), cyanogenic glycosides which are found in food such as cassava are known to have toxic effects such as; growth retardation as well as tropical ataxic neuropathy Islamiyat *et al.*, (2016), chromone glycosides are used for their anti-ulcer, anti-inflammatory and anticancer properties Keri *et al.*, (2014), flavonoid glycosides are found to be useful antioxidants Hollman *et al.*, (1999), saponin glycosides are known to possess cytotoxic and antiulcer properties Ozlem and Giuseppe, (2007), thioglycosides such as lincomycin A are being utilized for their antibiotic properties Lian *et al.*, (2014). Terpenoids are secondary metabolites consisting of 5 member isoprene units which are linked together and they have good anti-inflammatory properties Villar *et al.*, (2003).

Medicinal plants are known to be rich in mineral elements such as calcium, magnesium, iron, and potassium. These elements are essential for plant biosynthetic and metabolic processes. They also contribute to the pharmacological and nutritional properties of the plants. The nature and amount of mineral elements present in a particular plant are dependent on the type of soil it was cultivated as well as the geographical region. Sometimes, plants may contain essential minerals in very high concentrations which may be toxic when consumed or may also contain heavy metals such as lead, which is toxic Jasper *et al.*, (2017).

The rich components of medicinal plants which contribute to their biological activity have attracted the attention of various researchers seeking to discover new drugs with better activity with little or no side effects compared to the currently existing drugs Maridass and Britto, (2008). Many drugs used today are derived from plants. Examples of such drugs are; quinine obtained from *Cinchona species*, digoxin from *Digitalis lanata*, codeine from *Papaver somniferum* and pilocarpine from *Pilocarpus jaborandi* Maridass and Britto, (2008). Many newer agents derived from plants are still being manufactured today as a result of research conducted to explore the potentials of these medicinal plants. Drugs like; paclitaxel, artemisinin, etoposide, and mevastatin are examples of newer medicines derived from plants Maridass and Britto, (2008).

Eleusine coracana (EC) also popularly known as finger millet belongs to the family Poaceae found mainly in the arid regions of Asia and Africa where they are used as staple food especially by the low-income earners Glew *et al.*, (2008). Several studies have been carried out on EC, especially on the seeds and leaves extracts and isolated compounds, and these have exhibited promising pharmacological results (Osgdale *et al.*, 2017). Consumption of the flour made from the seeds of the plant and its organic solvents extracts have been shown to be beneficial in the management of diabetes, obesity, reduction of cholesterol levels, liver disorders, prevention of osteoporosis, ulcer, Mall and Tripathi, (2016); Devi *et al.*, (2014); Denish *et al.*, (2016). Extracts of EC have demonstrated strong antioxidant potentials, Mathanghi and Sudha, (2016); Singh *et al.*, (2015); Sreeramulu *et al.*, (2009), anticancer

activity against HepG2 liver cancer cell lines, Singh *et al.*, (2015) and K562 cancer cell lines, Sen *et al.*, (2011); antimicrobial activities, Shukla *et al.*, (2015) anti-aging activity, Hedge *et al.*, (2002), wound healing and antilithiatic effects, Mathanghi and Sudha, (2012). It was also shown to exhibit anti-cataract effects, Chethan *et al.*, (2008) and Shobana *et al.*, (2010), and also hepatoprotective activity, Pingle *et al.*, (2011). The plants and its products have been employed as nutraceuticals in recent times.

This study aims to determine the phytochemical and mineral contents in the seeds of *Eleusine coracana* (EC) which may contribute to its pharmacological activities; establish its quantitative parameters for authentication and the acute toxicity profile to ascertain its safety level. This is with the focus of providing nutraceutical companies and users concise information on the nutritional status and safety information on *Eleusine coracana* potentials as a health food.

MATERIALS AND METHODS

Plant collection and preparation

Eleusine coracana seeds were purchased from a local market in Jos Plateau, Nigeria in June, 2016. It was identified at the Forestry Research Institute of Nigeria and was assigned a voucher specimen number FHI 59920. The seeds were air-dried for a week, pulverized and stored in a closed glass container until further use.

Quantitative parameters determination of powdered seeds of EC

The quantitative parameters of the powdered sample of EC were determined through proximate analysis using standard methods described in the African Pharmacopoeia (1986) and AOAC (1984).

Moisture content/Water loss on drying

Two grams of the powdered seeds of *Eleusine coracana* was weighed into a crucible previously cleaned, dried and weighed. The crucibles and its contents were dried in an oven at a temperature of 105°C until a constant weight was obtained. The procedure was repeated for six replicates and the average percentage weight loss or percentage moisture content was determined, African Pharmacopoeia (1986); AOAC (1984).

$$\text{Moisture content (\%)} = \frac{(\text{weight loss})}{(\text{Initial Weight of Sample})} \times 100\%$$

Total ash

Twelve crucibles were washed and placed in an oven set at 100°C. The crucibles were marked 1-12 while still warm. They were transferred into and allowed to cool in a desiccator and subsequently weighed. Two grams of the powdered seeds were weighed into each of the dried crucibles. The crucibles and its powdered content were incinerated in a muffle furnace set at 600°C for 6 hours after which the furnace was switched off and the temperature allowed to drop. The crucibles were brought out and allowed to cool in a desiccator and reweighed. The

percentage of ash was calculated for six replicates, African Pharmacopoeia (1986); AOAC (1984).

$$\text{Percentage Total Ash (\%)} = \frac{(W - Z)}{N} \times 100\%$$

Where; W = Weight of the crucible and ash, Z = Weight of empty crucible and N = Weight of sample.

Acid-insoluble ash

Ash obtained from the total ash experiment above was transferred into a beaker. 25 mL of dilute acid (HCl) was added and the resulting mixture was boiled for 5 minutes over a water bath. An ashless filter paper was used in filtering the boiled mixture and the ashless filter paper containing the residue was folded into a cone shape which was placed into a crucible and washed completely in a furnace. The crucible was allowed to cool in a desiccator and the weight of the residue was determined. Based on the initial weight of the air-dried sample, the percentage of acid insoluble ash was calculated. The determination was carried out in triplicates, African Pharmacopoeia (1986); AOAC (1984).

$$\text{Acid insoluble Ash Value} = \frac{\text{Weight of Residue}}{\text{Initial Weight of the sample}} \times 100\%$$

Water soluble ash

The total ash contained in the remaining six crucibles from the total ash experiment was transferred carefully into six beakers each containing 25 mL of distilled water. The beakers were boiled over a water bath for 5 minutes after which, the resulting solution was filtered using an ashless filter paper. The filter paper containing the residue was folded into a small cone shape and returned to their respective crucibles. The crucibles were placed in a furnace where they were heated until a temperature of 600°C was attained and maintained for 6 hours. After the 6 hours, the crucibles containing the ash were allowed to cool in a desiccator and weighed. The water soluble ash value was calculated by subtracting the weight of the residue from the weight of the total ash. The difference obtained was expressed as a percentage of the initial weight of the sample, African Pharmacopoeia (1986); AOAC (1984).

$$\text{Water Soluble Ash Value} = \frac{\text{Weight of Ash} - \text{Weight of Residue}}{\text{Initial Weight of the sample}} \times 100\%$$

Alcohol soluble extractive value

Five grams of the powdered seeds of *Eleusinecoracana* was weighed accurately into a 250 mL conical flask and was macerated with 100 mL of 98% methanol for 24 hours. The content of the flask was shaken intermittently. Buckner funnel was then used in filtering and 20 mL of the filtrate was poured into a previously cleaned and dried crucible. The filtrate was allowed to evaporate to dryness until a constant weight was obtained which was recorded. The alcohol extractive value was calculated based on the initial weight of the powdered seed and the result obtained was expressed in percentage.

Water soluble extractive value

The above experiment of Alcohol soluble extractive value was repeated using chloroform water in the ratio of 1:400.

Phytochemical Screening of the powdered seed of *Eleusine coracana*

Phytochemical screening was carried out on the powdered seeds of EC using standard methods involving simple chemical tests Sofowora, (1982); Evan, (2002). The screening was carried out to determine the presence of secondary metabolites in the seeds such as; alkaloids, saponins, anthraquinones, phenolics, tannins, proteins and carbohydrates.

Five grams of the powdered seeds of EC was boiled in 75 mL of distilled water on a water bath for 30 minutes. The resulting solution was filtered using a filter paper while still hot and allowed to cool. The filtrate was used to carry out the following tests.

Mineral Analysis

Mineral analysis of the powdered sample was carried out according to standard methods described in the AOAC, (1990). The powdered material (1 g) was digested with 10 mL of a mixture of nitric acid (HNO₃) and perchloric acid (HClO₄) in the ratio 3:1 in a conical flask with heating until colour fumes disappeared. The digested material was filtered through a Whatman No 1 filter paper into a 100 mL volumetric flask and made up to mark with distilled. A flame photometer was used to analyze the sample for sodium and potassium while an atomic absorption spectrophotometer was used to analyze for the presence of other elements AOAC, (1990).

Acute Toxicity Study

Animals

Animals used to carry out this study were adult swiss mice obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. Ethical approval with reference number EC/FP/018/26 was obtained from the Ethics Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Standard rodent pellets (Bendel feeds and Flour Mill Ltd, Ewu, Nigeria) were used to feed the animals which were also given water *ad libitum*. The animals were exposed to adequate lighting conditions and handled according to protocols that were approved by the faculty of pharmacy ethical committee, University of Benin.

Test Procedure

The study was carried out using the method described by Lorke (1983). The experimental protocol is in two phases.

Phase 1

Nine animals were used in this phase of the study. The animals were divided into three groups of three animals per group. The animals in the first group were administered 10 mg/kg body weight (bw) per oral dose of the crude extract, the second group of animals was administered 100 mg/kg bw dose of crude extract and the third group was administered 1000 mg/kg bw dose of the crude extract. The animals were monitored for 24 hours to detect the occurrence of behavioral changes and mortality.

Phase 2

Three animals were used for this phase and were shared into three groups of one animal each. The animals were given higher doses (1600, 2900 and 5000 mg/kg bw per oral) of the extract and observed for 24 hours and then every day within a 14 day period for behavioral changes and mortality.

The lethal dose was computed from the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

Statistical analysis

Results were expressed as mean \pm SEM. One way analysis of variance was used for result analysis using Graph Pad Prism software version 6 were applicable.

RESULTS

Proximate Analysis

The result of the proximate analysis is as shown in table 1. It revealed that the moisture content of the powdered seed of *Eleusine coracana* was very low and the water/chloroform extractive value was higher than the alcohol extractive value of the powdered seed.

Table 1: Proximate parameters of *Eleusine coracana* seeds.

Parameter	Value \pm SEM (%)
Moisture content	0.38 \pm 0.14
Total ash	2.23 \pm 0.13
Acid insoluble ash value	0.76 \pm 0.05
Water soluble ash value	0.01 \pm 0.01
Alcohol extractive value	0.21 \pm 0.03
Water/ chloroform extractive value	1.20 \pm 0.08

Phytochemical Screening

The phytochemical screening carried out on the aqueous extract of the powdered seed of *Eleusine coracana* as shown in table 2, revealed the presence of carbohydrates, alkaloids, proteins, reducing sugars, saponins, tannins, phenols, flavonoids, and terpenoids.

Table 2: Phytochemical constituents of *Eleusine coracana*

Phytochemicals	Inference
Carbohydrates	+
Alkaloids	+
Proteins	+
Reducing sugars	+
Saponins	+
Tannins	+
Phenols	+
Flavonoids	+
Terpenoids	+
Anthraquinones	-
Deoxysugars	-

+ represents the presence of components

- represents the absence of components

Elemental Analysis

The result of the elemental analysis in table 3 showed that sodium, potassium, calcium, magnesium, zinc, iron, copper and chromium are present in the seeds of EC. Nickel and cadmium were absent while a very low concentration of lead (0.01) was detected.

Table 3: Elemental composition of *Eleusine coracana*

METALS	CONCENTRATION (PPM)	WHO LIMIT (PPM)
Sodium (Na)	11.66±1.17	-
Potassium (K)	19.72±0.39	-
Calcium (Ca)	362.20±9.01	-
Magnesium (Mg)	1.06±0.00	-
Zinc (Zn)	0.35±0.01	27.40
Cadmium (Cd)	0.00±0.00	0.21
Lead (Pb)	0.01±0.00	10.00
Iron (Fe)	2.84±0.10	20.00
Copper (Cu)	0.19±0.01	3.00
Nickel (Ni)	0.00±0.00	1.63
Chromium (Cr)	1.23±0.07	-

Acute Toxicity Studies

The experimental animals were observed for signs of toxicity (lethargy, twitching, anorexia, seizures, excessive salivation) and death visually within a 24hrs period and then every day for 14 days post treatment with the crude extract.

The acute toxicity studies showed that oral doses of 10, 100, 1000, 1600, 2500 and 5000 mg/kg body weight, respectively of the crude extract of EC produced no toxic effects on the animals and no mortality was recorded even after two weeks of observation.

DISCUSSION

Proximate analysis carried out on the powdered seeds of *Eleusine coracana* showed very low moisture content (0.38 ± 0.14%) which is below the 6-8% limit set by the African Pharmacopoeia, (1986). This low moisture content implies that the seeds

can be stored for a long period without fear of microbial degradation and moisture-induced hydrolysis of active principles. Hence a powder formulation of the seed would possess a reasonable shelf life. We observed that the moisture content valued obtained in this study varied remarkably from a previous study conducted by David *et al.*, (2014) who gave a moisture content of 6.99%. The ash value which is a representation of the mineral or inorganic constituent of the seed after water and organic components have been removed by heat was found to be 2.23 ± 0.13%. This is consistent with the ash value (2.37%) previously reported by David *et al.*, (2014). The water soluble ash which shows the percentage of minerals present in the plant that is soluble in water was found to be 0.01 ± 0.01%. And it was also observed that the chloroform/water extractive value (0.21 ± 0.03 %) was higher than the alcohol extractive value (1.20 ± 0.08 %) indicating

that the mixture of a polar and non-polar solvent is better able to extract the constituents (polar and non-polar constituents) of the seeds than using an organic solvent like alcohol alone.

The phytochemical analysis carried out on the powdered seeds of *Eleusine coracana* showed the presence of important phytochemicals such as carbohydrates, alkaloids, proteins, reducing sugars, saponins, tannins, phenols, flavonoids and terpenoids which contribute to the pharmacological activities generally observed in medicinal plants. The fact that these phytochemicals are present indicates that the seeds of *Eleusine coracana* may have some important pharmacological activities that need to be explored. Anthraquinones and deoxysugars were found to be absent. However, the results obtained from this phytochemical screening were different from those reported by Pingle *et al.*, (2011), who reported the absence of alkaloids in the seeds.

Results obtained from the elemental analysis to determine the presence of heavy metals and also essential and non-essential minerals showed that heavy metals such as; cadmium (Cd) and nickel (Ni) were absent in the sample far below the WHO allowable limits (0.21 – 1.60 ppm), while lead (Pb) was present in a negligible concentration (0.01 ± 0.00 ppm). This shows that the seeds are safe for consumption and does not have the potential of causing health hazards associated with the consumption of foods and food products containing a high concentration of heavy metals. The analysis also revealed the presence of essential minerals such as; calcium (362.20 ± 9.01 ppm), iron (2.84 ± 0.10 ppm),

potassium (19.72 ± 0.39 ppm), sodium (11.66 ± 1.17 ppm) and magnesium (1.06 ± 0.00 ppm) which are important for normal biological functions in the body Soetan *et al.*, (2010) and also take part in the pharmacological activities expressed in medicinal plants Jasper *et al.*, (2017). Some trace elements detected in the powdered seeds are copper (0.19 ± 0.01 ppm), zinc (0.35 ± 0.01 ppm) and chromium (1.23 ± 0.07 ppm) which are known to be components of enzymes and also participate in normal biological processes in the body Wada, (2004). The indication of this is that the consumption of EC would help resolve health issues associated with the deficiency of certain essential and non-essential minerals. The results from the acute toxicity experiment show that the extract of EC is safe at the dose ranges used and therefore very safe for consumption.

CONCLUSION

Eleusine coracana seeds also known as finger millet is a promising seed that has shown good phytochemical and mineral profile. The presence of useful phytoconstituents and essential minerals is an indicator that these seeds may have great potential in the management of different illnesses. The acute toxicity profile and the absence of heavy metals show that the seeds are safe and could be formulated as nutraceuticals for the management of diseases.

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