African Scientist Vol. 20, No. 1, March 31, 2019
Printed in Nigeria

1595-6881/2019 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2019054/20103

Preliminary Studies on *Physostigma venenosum* (Balf.) Seeds: Mineral Composition, Proximate and Phytochemical Analysis

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(Received November 17, 2018; Accepted in revised form January 20, 2019)

ABSTRACT: *Physostigma venenosum* Balf., known as Calabar beans (Fabaceae family) is a very poisonous plant with reported therapeutic properties. Many studies have shown its use in medicine for eye problems, glaucoma, constipation, epilepsy, cholera, hypertension and tetanus. In this study, quantitative phytochemistry, proximate analysis and mineral constituents of the ethanol extracts of *Physostigma venenosum* seeds were investigated using standard analytical chemical procedures. Phytochemical analysis revealed the presence of secondary metabolites like alkaloids, saponins, tannins, oxalate and phytate with their corresponding values as 96.11, 1.08, 2.16, 36.56 and 12.8 mg/100g respectively. Proximate analysis was carried out to determine the nutrient contents of the sample. The results revealed the following data: crude lipid (3.21 %), crude fibre (4.08 %), crude protein (70.88 %), ash (6.04 %) and carbohydrate (13.85 %). Various minerals were present with high concentrations of sodium, calcium and potassium contents. It can be deduced from the results of this study that *P. venenosum* contained appreciable amounts of phytochemicals, nutrients and minerals that aid its medicinal properties.

Keywords: *Physostigma venenosum*, phytochemical analysis, proximate composition, mineral contents, ethanol extracts.

Introduction

Physostigma venenosum Balf, commonlyknown as Calabar beans belongs to the family Fabaceae. It is widely distributed in tropical Africa, particularly in the Old Calabar region of Nigeria where it is reported to have originated. *P. venenosum* is an herbaceous perennial legume that thrives mainly on the banks of streams and produces seeds that are mostly abundant during the rainy season in Africa between June and September (Scheindlin, 2010). It is a twining climber with pinnately trifoliate leaves, pendulous racemes of purplish bean-like flowers. The seeds are enclosed ina dark brown pod of about 6 inches long and are kidney-shaped. They are thick, about 1 inch long with rounded ends. The seeds are roughish but a little polished and have a long scar on the edge at the point of attachment to the placenta.

Over the years, *Physostigma venenosum* had gained prominence in Africa following the use of its seeds as an “ordeal poison” to ascertain if a person was a witch or possessed by evil spirits (Laura, 2003).Although, the whole seed is highly poisonous, but with a number of medicinal applications. Ethnobotanically, it is used as laxatives, anticonvulsants, antibiotics, antimicrobial agents and anti-epileptic drugs. However, when used in excess, it causes muscular weakness, respiratory failure and cardiac arrests (Bown, 1995). In recent time, *P. venenosum* which was originally consumed in African ritual ordeals has been shown to produce phytochemicals relevant for the treatment of eye diseases, as it causes rapid contraction of the pupil (Miguel *et al.*, 2012). Also, toxicological studies of the seed extract have shown its relevance as an immune system booster at graded dose level (Aihiokhai *et al.*, 2017).Wickersham and Novak (2003) implicated its use in chronic constipation as a stimulant to unstrap intestinal muscles and its action on the circulatory system to slow pulse and raise blood-pressure.

The use of *P. venenosum* for therapeutic purposes is premised on the reported isolation of physostigmine, a reversible cholinesterase inhibitor alkaloid that is rapidly absorbed through membranes and applied topically to the conjunctiva for the treatment of glaucoma and also used to treat the central nervous system effects of atropine overdose, hallucinations, arrhythmias, hypertension, coma, myoclonic seizures and other anticholinergic drug overdoses (Scheindlin, 2010).

Ramalingam *et al.* (2010) stated that phytochemicals are chemicals that are present naturally in plants. Today, these phytochemicals became more popular due to their countless medicinal uses, as they play vital roles against a number of diseases such as asthma, arthritis, cancer etc. than pharmaceutical chemicals since they have little or no side effects (Afolayan *et al.*, 2008).

According to the World Health Organization, medicinal plants are plants that are employed in the control or treatment of diseases as a result of the chemically active components they contain. The parts of such plants used for therapeutic purposes include the leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds. These bioactive components are often referred to as phytochemicals or phytoconstituents (Nweze *et al*., 2004; Doughari *et al*., 2009). Most of these phytochemicals have been isolated and characterized from fruits, vegetables, spices, beverages, as well as many other sources (Doughari & Obidah, 2008; Doughari *et al*., 2009).

Koleva *et al*. (2002) reported that phytochemical analysis can be used as a basis for screening for plants antioxidant properties. It has been shown that phytochemical analysis aids in tracing plants constituents (Basu *et al.,* 2013). Phytochemical screening carried out by Arote *et al*. (2009) on *Pongamia pinnata* indicate the presence of secondary metabolites such as flavonoids, saponins, tannins and alkaloids. It revealed that dietary supplements can be used to fight or prevent some common ailments.

Human beings require food to carry out essential functions, which includes growth, development and reproduction (Bradbury, 1998). Some plants provides nutrient required for energy, bodybuilding, maintenance and regulation of body processes. These nutrients include carbohydrates, lipids, proteins, minerals, vitamins and water (Risslin, 2001). The nutritional quality of foods may be evaluated by chemical analysis of the food for proximate composition, mineral content, vitamin content, amino acid profile of the protein, fatty acid profile of the lipid and levels of anti-nutritional substances (Noecker, 2006). Leland (2006) revealed that *Dioscorea spp*. and *Manihot esculenta* are eaten mainly as sources of energy since they are all very high in total carbohydrate content when compared to their low protein content. Minerals are considered essential to man as they provide basic macro and micro nutrients. The macro mineral nutrients are calcium, chlorine, phosphorus, sulphur, sodium, potassium and magnesium; while zinc, nickel, fluorine, iron, copper, manganese, selenium, molybdenum, chromium, tin and cobalt constitute the micro mineral nutrients. However, this plant species has been validated to be poisonous and with great therapeutic potential. Hence, to investigate the active constituents for its therapeutic importance, the present study was carried out for quantitative phytochemical analysis, proximate and mineral composition of the seeds of *P. venenosum* using ethanol extract.

Materials and methods

Seed source and processing: The seeds of *Physostigma venenosum* were obtained from a local market in Calabar metropolis in Cross Rivers State of Nigeria. The seeds were purchased dried and further subjected to drying in an oven (60^0C) for complete dehydration. After dehydration, 200 g of the dried samples were crushed using a blender to produce a fine composite powder. Extraction was carried out by soaking one hundred grams (100 g) of the powdered sample in 200 ml of absolute ethanol for 48hours in a glass container. After filtration, a rotary evaporator was used to concentrate the extract from which various assays were conducted.

Phytochemical analysis: The quantitative chemical constituents of *P. venenosum* seeds were investigated for tannin, saponin, alkaloids, oxalate and phytates using standard methods of Trease and Evans (2002), Obadoni and Ochuko (2001), Harborne (1998), Day and Underwood (1991) and UV/visible spectrometric method respectively.

Proximate analysis: The recommended methods of the Association of Official Analytical Chemists (AOAC, 2005) were used for the determination of crude lipid, crude fibre, crude protein,ash and carbohydrate content of the samples. The respective determinations were done in triplicates.

Mineral analysis: The mineral elements of *Physostigma venenosum* seeds: sodium (Na), potassium (K), calcium (Ca), magnesium(Mg), iron (Fe), zinc (Zn), manganese (Mn) and lead (Pb) were determined using the atomic absorption spectrophotometer (AAS-Buck 205), asoutlined in the methods of the Association of Official Analytical Chemists (AOAC, 2005). All the determinations were done in triplicates.

Statistical analysis: The data obtained from this study were analyzed using descriptive statistical tools and presented as mean ± standard error of mean (SEM) of three replicates (mean ± SEM). The proximate analysis was expressed in percentage (%) while the phytochemical and mineral contents were expressed in mg/100g and mg/kg respectively.

Results

The results are presented in three (3) parts, namely: quantitative phytochemical analysis, proximate analysis and mineral composition of the plant.

Phytochemical analysis: The result of the quantitative determination of the phytochemical constituents reveals high alkaloid content and low saponin content of 96.11 mg/100g and 1.08 mg/100g respectively as shown in Table 1.

Table 1: Phytochemical composition of the ethanolic extract of the seeds of *P .venenosum* expressed in mg/100g

Parameters	**Concentrations (mg/100g)**
Tannin	2.16 ± 0.65
Saponin	1.08 ± 0.52
Alkaloid	96.11± 0.36
Oxalate	36.56 ± 0.63
Phytate	12.80 ± 0.45

Data presented as Mean ± SD; n = 3

Proximate analysis: Proximate analysis of the seeds of *P. venenosum* reveals a significantly high protein content of 70.88± 0.96% when compared with other nutrients as presented in Table 2.

Table 2: Proximate composition of the ethanolic extract of the seed of *P. venenosum*

Parameters	**Concentration (%)**
Crude lipid	3.21 ± 0.27
Crude fiber	4.08 ± 1.00
Crude protein	70.88 ± 0.96
Ash	6.04 ± 0.08
Carbohydrate	13.85 ± 0.44

Data presented as Mean ± SD; n = 3

Mineral composition: Table 3 shows the mineral composition of *P. venenosum* seeds. The result recorded high sodium, potassium and calcium contents of 605.86, 519.46 and 427.51 mg/kg respectively.

Table 3: Mineral composition of *P. venenosum* seeds

Minerals	**Concentration (mg/kg)**
Sodium	605.86
Manganese	38.21
Potassium	519.46
Calcium	427.51
Magnesium	321.86
Lead	5.36
Zinc	94.89
Iron	146.34

Discussion and conclusion

The results obtained shows that the seeds of *Physostigma venenosum* consist of secondary metabolites such as tannins, alkaloids, saponins, phytate and oxalate. These metabolites have been implicated to be responsible for the therapeutic properties of plants (Vishnu *et al.*, 2013). The alkaloid content of *Harungana madagascariensis* (2.45±0.42 mg/100 g) seeds reported by Fredrick and Osamede (2014), is comparable to that of *Aframomum melegueta* (2.45±0.42 mg/100 g) seeds extracts (Echo *et al.* 2012), but considerably lower than that of *P. venenosum* (96.11± 0.36 mg/100g) recorded in the current study. This high alkaloids concentration of *P.*

venenosum supports its use as a central nervous system stimulant, topical anaesthetic in ophthalmology, powerful pain relievers, antipruritic action, among other uses (Aja *et al.,* 2010).It also implies that alkaloids are considered poisonous when large quantity is taken due to their stimulatory effect that produces excitation peculiar to cell and nerve disorders (Obochi *et al.,* 2006). However, the class of alkaloids, physostigmine present in this plant is among the powerful poisons known (Ehimwenma *et al.,* 2007). It stimulates most involuntary muscles in the body and it is mainly used for the treatment of glaucoma due to its extreme pupillary contraction (Adebisi *et al.,* 2013). The presence of tannins shows that the seeds of this plant could be used as a treatment for constipation and tetanus (Sodipo e*t al.,* 2001). The phytate content (12.80 ± 0.45) of the seeds is lower than that of *Danniellia oliveri* kernel seed 51.05 mg/100 g (Hassan *et al.,* 2008) and higher than that of *Jatropha curcas* kernel seed 34.37 mg/100g (Azza *et al.,* 2010). It has been reported that increased phytate content can result in binding of some essential mineral nutrients in the digestive tract, thereby decreasing the bioavailability of minerals in organisms (Bello *et al.,* 2008). Phytate has also been shown to have immense antioxidant effect (Ali *et al.* 2010), while saponins are glycosides of both triterpene and steroids having hypotensive and cardiode pressant properties (Olaleye, 2007). Adesokan and Akanji (2010) reported the anti-carcinogenic and antimalarial properties associated with saponins. Hence, *P. venenosum* may be suitable for these therapeutic purposes.

The proximate composition of *P. venenosum* as presented in Table 2, shows that crude lipid recorded the lowest (3.21 ± 0.27%) amongst the nutrient composition while crude protein was found to be the highest (70.88 ± 0.96%).Legumes, except the oil legumes, have been reported to be low in fat content ranging from 1-5%. However, oilseeds have a range of lipid contents from about 18% in *Glycine max*to as high as 43% in *Arachis hypogeae* (Dangoggo, *et al.,* 2011; Hassan *et al.,* 2008). This implies that *P. venenosum* cannot probably be said to be an oil seed. Lipids are very essential because they provide the body with maximum energy (Oluyemi, *et al.,* 2006). Of most legumes, *Glycine max* are among the richest in terms of protein content (43%), while others have protein content ranging from 20 - 25% as reported by Jimoh and Oladiji (2005). The exceptionally high protein content of *P. venenosum* seeds (70.88 ± 0.96%) when compared with that of *Glycine max* (43%) as attributed to studies of Jimoh and Oladiji, (2005), may have contributed to its therapeutic use for the management of some systemic and nutritionally related diseases. The ash content of the studied seeds was 6.04 ± 0.08%, which was relatively similar to the findings of Aremu *et al.* (2006) on *Anarcadium occidentale* flour. It has been recommended that ash contents of nuts, tubers and seeds fall in the range of 1.5-2.5% in order to be suitable for animal feeds (Akinhanmi, 2008). The ash content of *P. venenosum* does not fall within this range hence, it cannot be recommended for animal feeds. The crude fibre of the seeds is 4.08 ± 1.00% which compared favourably to legumes with mean values ranging from 5-6% (Aremu *et al.* 2006). Although fibre containing seeds are known to enhance digestibility by easing the passage of waste, thus functioning as an effective anti-constipation, and also lowers blood cholesterol level, thereby reduces the risk of various cancers. But emphasis has been placed on the importance of keeping fibre intake moderate because the presence of high fibre levels in diet can cause intestinal irritation, lower digestibility with an overall decrease in nutrient utilization (Bello *et al.,* 2008). The available carbohydrate content of the seeds was found to be 13.85 ± 0.44%. When compared with conventional sources of carbohydrates like cereals with 72-90 mg/100g carbohydrate as reported by Adewusi *et al.* (2005). Hence, the seeds of *P. venenosum* could not be considered as a potential source of this nutrient.

The mineral composition of the seeds of *P. venenosum* (Table 3) was found to have high sodium (605.86 mg/kg), potassium (519.46 mg/kg), calcium (427.51 mg/kg) and magnesium (321.86 mg/kg) contents when compared to the manganese (38.21 mg/kg), zinc (94.89 mg/kg) and lead (5.36 mg/kg) contents. This indicates that the seeds of *P. venenosum* have high concentration of macronutrients than the trace elements (micronutrients). These results are in contrast with the observation of Mutayoba *et al.* (2011) and Aremu *et al.* (2006). Minerals are necessary for normal growth, skeletal and muscular activities (such as calcium), intestinal absorption and metabolic activities in the body (such as magnesium), oxygen transport and cellular activities (copper and iron), regulation of acid-base balance (phosphorus), as well as nerve transmission and fluid balance (sodium and potassium) as reported by Oluyemi *et al.* (2006). Iron aids in prevention of anemia, manganese plays arolein supporting the immune system as well as energy production and zinc is essential in protein and carbohydrate metabolism and also help in mobilizing vitamin A from its storage site in the liver, thereby enhancing the synthesis of nucleic acids relevant for cell production. Magnesium, calcium and phosphorus are major constituents of bones and teeth, but magnesium is important in tissue respiration as it leads to the formation of Adenosine triphosphate(ATP) and its deficiency results in the abnormal twisting of the muscles leading to convulsion and tetanus (Muhammad *et al.,* 2011).

In conclusion, the results obtained from the phytochemical analysis of the extract has shown the presence of some secondary metabolites which indicates that the plant may possess immense medicinal value. The plant has been shown to contain relatively rich composition of nutritional properties, especially protein and carbohydrate contents as well as minerals that are essential to man, which suggests that it may be useful to industries for the commercial exploitation with pharmaceutical and nutraceutical attributes to herbal formulations. Nutrient analysis also suggests that this medicinal plant can be used for preparation of herbal products and standardized extracts.

Further research would be desirable, to determine the possible toxicants that may be present in the seeds of this plant for which it is not safe for direct human consumption despite its rich nutrient and therapeutic potentials.

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