In-Vitro Bioavailability Evaluation of Selected Mycotoxins, Antinutritional Factors and Vitamin in Stored *Ricinus Communis* Seed Powder

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ABSTRACT

Ricinus communis seeds are susceptible to fungal contamination when exposed. This study assessed the *in vitro* bioavailability of aflatoxins, anti-nutritive factors and vitamins in stored *Ricinus communis* seed flour. Fresh *Ricinus communis* seeds were collected, dehulled, air dried and ground to powder. The ground sample was divided into two portions and exposed to open air in the laboratory for two weeks. Subsequently, one portion was macerated in distilled water for 72 hours at room temperature and the other portion was suspended in simulated gastric fluid (SGF) for 1 hour at 37^0C and aliquot samples collected at 15 minutes intervals for analysis. Aflatoxins content was analyzed using HPLC (Biobase model with UV–Vis spectrophotometer detection system) and anti-nutritive phytochemicals and vitamins content were analyzed using standard spectrophotometric methods. The results revealed the presence of Aflatoxin B1 (AfB1), Aflatoxin G1 (AfG1), Aflatoxin G2 (AfG2), flavonoids, alkaloids, polyphenols, tannins cyanogenic glycosides and vitamin B12 at varying concentrations. Whereas the macerated extract contained AfB1, AfG1 and AfG2 at various levels, AfB1 was not detected in the SGF extract. Tannins and cyanogenic glycosides in the macerated extract and SGF were above 5.80 mg/ml and 0.90 mg/ml respectively and the levels increased with time in the SGF. Vitamin B12 was detected in the SGF extract. These results suggest that whereas AfG1, AfG2, tannins, cyanogenic glycosides and vitamin B12 in the stored *Ricinus communis* seed powder were bioavailable in SGF, the carcinogenic AfB1 was not detected in the SGF.

Keywords: *Ricinus communis*, Aflatoxins, Simulated gastric fluid, Anti-nutritive factor, vitamin

INTRODUCTION

Ricinus communis, also known as castor bean, is a valuable shrub found in Asia and Africa. Castor bean is a widely cultivated plant known for its seeds, which contain a variety of nutrients, including vitamins and fatty acids. Its fruit has a spiny skin with three lobes containing brown speckled oval seeds [1]. According to [2] *Ricinus communis* produces seeds that are rich in oil. The seed oil contains beneficial oleic, ricinoleic, and linoleic acids, among others, which are good for health [3]. Castor oil, extracted from the seeds, has various uses in pharmaceuticals, cosmetics, and industry [4]. Its medicinal applications include being used as a laxative, anti-fertility agent and skin disease conditions [5]. Industrially, it is used in the production of lubricants, paints, and biodiesel. Today, the oil is also found in a plethora of skin and hair products for its smoothening properties. However, it's essential to handle castor beans carefully as they contain ricin, a potent toxin.

Vitamins are essential non-caloric micronutrients which play crucial roles in various biochemical and physiological processes, including metabolism, immune function, and antioxidant defense [6]. The presence of vitamins in *Ricinus communis* seeds, such as vitamin E (tocopherols) and vitamin A (retinoids), and the B-complex vitamins, highlights their nutritional significance [7, 8].

Mycotoxins are toxic secondary metabolites produced by certain fungi that can contaminate food and feed in storage, posing risks to human and animal health. *Ricinus communis* seeds are susceptible to mycotoxin contamination from fungi of the genera *Aspergillus* and *Penicillium* under certain conditions, due to its rich oil content. Mycotoxins such as aflatoxin have been reported in castor seeds [9]. Aflatoxins and ochratoxins are of particular concern due to their carcinogenic and hepatotoxic effects [10].

Anti-nutritional factors (ANFs) are naturally occurring chemicals produced by plants and other food sources through various metabolic processes. These factors are produced by plants as a defense mechanism and many of them fall under the category of secondary metabolites [11]. Plants need ANFs to resist herbivores, insects, pathogens, and poor growth conditions. When consumed, these compounds reduce the bioavailability of nutrients in the body or interfere with the optimal use of certain nutrients like vitamins, proteins, and minerals. Oilseeds contain different anti-nutritional factors such as goitrogens, aflatoxins, phenolic compounds, phytic acid, lectins, and glucosinolate [12]. Consumption of cereals, legumes and oilseeds can lead to protein indigestibility due to the presence of these factors.

The presence and bioavailability of nutrients and xenobiotics in stored *Ricinus communis* seeds is of particular interest due to the potential impact on human and animal health. Bioavailability generally refers to the extent a bioactive molecule is soluble and absorbed into systemic circulation and utilized by the body. Understanding the bioavailability of vitamins, ANFs and mycotoxins in *Ricinus communis* seeds is essential for assessing their nutrient value, safety and potential health risks if consumed.

Therefore, this *in vitro* bioavailability evaluation of selected mycotoxins, ANFs and vitamin in stored *Ricinus communis* seeds aims to simulate gastrointestinal digestion process of these inherent biomolecules in the seed. This is essential in assessing their potential dissolution and release for intestinal absorption.

MATERIALS AND METHODS

Ricinus communis seeds from dehiscence mature capsules of the plants were obtained from Mile 3 market in Port Harcourt, Rivers State, Nigeria, and used for this research. The plant capsule and seed samples were identified at the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt. The *Ricinus communis* seeds were ground to powder using an electric blender to obtain the seed flour which was subsequently exposed to open air, at room temperature, in the laboratory for two (2) weeks. The ground sample was divided into two portions; 25g of one portion was weighed and macerated in 500 ml of distilled water for 72 hours at room temperature. After which the macerate was double filtered using Whatman No1 filter paper and the filtrate analyzed. The other portion was weighed, transferred onto a Whatman No1 filter paper and suspended in 500 ml of simulated gastric fluid (SGF) in a beaker placed on a temperature-controlled magnetic stirrer maintained at 37±0.5ºC with rotor speed set at 75rpm. Aliquots of the SGF were collected at 15 minutes intervals, over a period of 1hr, using a syringe. For every aliquot collected, equal volume of freshly prepared SGF was added to maintain the total operating volume of 500ml. The SGF was prepared according to the method recommended by [13].

Analysis of aflatoxins, anti-nutritional factors and vitamin B12 content of the extracts were done using standard methods. The Aflatoxins content was determined using HPLC method as described by [5], Anti-nutritional factors (ANFs) was determined using standard spectrophotometric methods as described by [3] and Vitamin B12 was determined using the method described by [14].

Statistical Analysis

The statistical analysis of the results from the study was done using Microsoft Excel office and values were expressed as mean values ± standard deviation of triplicate values obtained.

RESULTS

Results of Aflatoxins and Vitamin B12 levels in stored *Ricinus communis* seed powder extracts are presented in Table 1. Whereas Aflatoxin G1 (AfG1) Aflatoxin G2 (AfG2) and Vitamin B12 were detected in the SGF extracts, Aflatoxin B1 (AfB1) was present in the distilled water (aqueous) extract and not detected in the SGF extract. Vitamin B12 was detected in SGF extract but not detected in the aqueous extract

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Table 1: Levels of Aflatoxin and Vitamin B12 in stored Ricinus *communis* seed powder extract

Bioactive compounds	Concentration DWE	(µg/L) SGF
AfB1	29.1687 ± 0.001	ND
AfG1	0.1333± 0.003	2.5440± 0.001
AfG2	0.2607± 0.001	1.2505 ± 0.002
Vitamin B12	ND	39.00 ± 0.001

*Values are mean of triple determination ± Standard deviation; DWE = Distilled water (aqueous) extract; SGF = Simulated gastric fluid; ND = Not Detected

The release profile of *in vitro* bioavailable (dissolved) phytochemicals in the *Ricinus communis* seed powder is presented in Figure 1. Alkaloids, Polyphenols, Tannins, Flavonoids and Cynogenic glycosides were detected at varying concentrations. Phytates and Oxalates levels were below detectable limits.

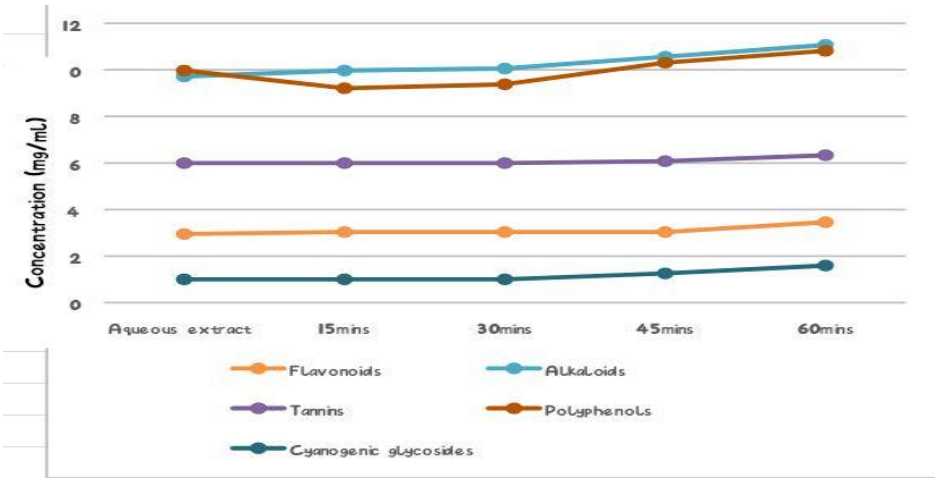

Figure 1: *In vitro* bioavailability profile of phytochemicals in stored *Ricinus communis* seed powder

The relative *in vitro* bioavailability (dissolved content) of tannins and cynogenic glycosides from stored *Ricinus communis* seed powder suspended in SGF and distilled water are presented in Figure 2. There was a marginal time-dependent increase in their dissolved levels with tannins showing higher values (more bioavailable) compared to cynogenic glycosides at each time interval

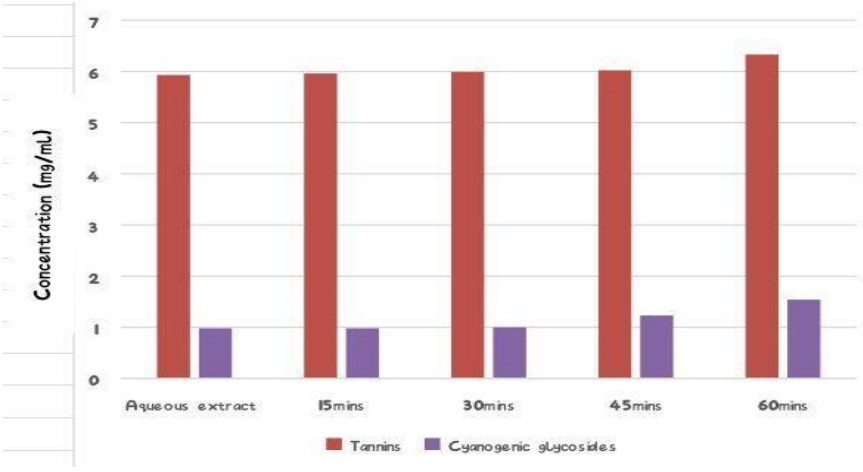

Figure 2: Levels of *in vitro* bioavailable tannins and cynogenic glycosides in stored *Ricinus communis* seed powder extracts

DISCUSSION

Phytochemical analysis of the *Ricinus communis* (RC) seed extracts revealed a diverse array of bioactive compounds, notably flavonoids, tannins, alkaloids, polyphenols and cyanogenic glycosides, which have established pharmacological and therapeutic applications. This is consistent with [15] who reported the presence of flavonoids, cyanogenic glycosides, saponins, oxalates, phytates, alkaloids and tannins in *Ricinus communis* seed oil.

Anti-nutritional factors such as tannins and cyanogenic glycosides have been reported to elicit toxic effects in both human and animal models. Tannins interfere with the digestion of many food substances leading to poor absorption of important nutrients [16]. Tannins bind strongly to proteins and form complexes which inhibit the activity of many digestive enzymes leading to low availability of proteins [17].

The relative low levels of oxalates and phytates in this study is beneficial with respect to nutrient absorption and utilization. Consumption of foods containing high levels of these anti-nutritional factors may cause toxic effects *in vivo*. Oxalate hinders the absorption of calcium ion resulting to unavailability of the calcium for various functions in the body. Oxalates binds to plasma calcium ions to form insoluble calcium oxalate complexes which accumulate as kidney stones. It has been reported that high levels of oxalate in foods causes irritation in the mouth and the lining of the gut [18]. Phytates bind strongly to several minerals including calcium, copper, iron, zinc, magnesium and molybdenum forming insoluble complexes that are poorly absorbed from the gastrointestinal tract leading to lower bioavailability of minerals in the body [19, 20]. The chelating property of phytate renders it a most effective anti-nutrient in foods and cause deficiency of minerals in animal and human nutrition [21]. Consumption of high phytate rich foods causes serious effects in infants, pregnant and lactating women [22]. Phytates also inhibit the activity of digestive enzymes including amylase, pepsin and trypsin [23]. The insignificant levels of oxalates and phytates in this study are consistent with earlier report [24]

In Table 1 three forms of aflatoxin were detected. Whereas AfG1 and AfG2 were detected in both samples, AfB1 was only detected in the macerated extract at 29.1687µg/L. This provides insight to the stability and degradation of aflatoxins in different environments. The detection of AfB1, AfG1 and AfG2 suggests that the stored RC seed powder may be contaminated with multiple aflatoxins [25]. A significant finding from the study was the presence of the widely reported carcinogenic AfB1 in the macerated extract but not in SGF extract, suggesting that AfB1 present in the stored seed powder may be degraded in the acidic and slightly elevated temperature conditions prevalent in the SGF. This is in tandem with the report in [25] where they reported that fungi actively grow on powdered plant materials containing large amount of lipids. However, in the RC seed powder, aflatoxin production was relatively low suggesting that some phytochemical constituents in the RC seeds may possess antimicrobial effect and inhibit aflatoxin production or they may have potential application in AfB1 detoxification. The detection of AfG1 and AfG2, two relatively unpopular variants of the aflatoxin mycotoxin, is instructive and deserve further investigation. Both variants were stable in the simulated gastric fluid condition.

Vitamin B12 was detected in the SGF extract at 39.00 ± 0.001µg/L. This is instructive as Vitamin B12 is not readily found in plant sources. However, it is possible that the stored seed powder may contain certain Vitamin B12 producing microbes like *Bacillus*, *Streptomyces*, *Pseudomonas* and *Archaea.*

CONCLUSION

This study investigated the *in vitro* bioavailability of selected mycotoxins, anti-nutritive factors and vitamins in stored *Ricinus communis* seed powder, by evaluating the dissolution profile of the biomolecules in simulated gastric fluid. The results indicates that the aqueous extract of stored *Ricinus communis* seed powder contain Aflatoxin B1, Aflatoxin G1 and Aflatoxin G2 as well as Tannins, Cyanogenic glycosides and Vitamin B12 at various concentrations. Distilled water extract contained Aflatoxin B1, Aflatoxin G1 and Aflatoxin G2 while simulated gastric fluid (SGF) extract contained Aflatoxin G1 and Aflatoxin G2 mycotoxins only. Levels of dissolved (bioavailable) Tannins and Cyanogenic glycosides in both extracts were above 5.80 mg/ml and 0.90 mg/ml respectively. The levels increased with time in the simulated gastric fluid.

These results suggest that the unique condition of low pH, warm temperature and enzyme in the SGF decreased the bioavailability of the carcinogenic Aflatoxin B1 while these conditions did not significantly affect the bioavailability of Tannins, Cyanogenic glycosides and Vitamin B12.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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