

NUTRITIONAL COMPOSITION AND CYTOTOXICITY STUDIES OF Black Monkey (*Strychnos madagascariensis*) RIPE FRUIT

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ABSTRACT

Strychnos madagascariensis also known as black monkey orange is found in tropical and subtropical Africa including South Africa, Lesotho, Zimbabwe, and Swaziland. The fruit is consumed traditionally as food in the northern coastal region of Kwazulu-Natal in South Africa and the Southern part of Zimbabwe. This study investigated the nutritional, anti-nutritional composition, and cytotoxicity of *S. madagascariensis* ripe fruit. Fruits were randomly selected, and the parameters of each experiment were measured in triplicates. The seed coat and fruit pulp were analysed for proximate, mineral and anti-nutrients (phytic and oxalic acid) composition using standard protocols. The cytotoxic effect of methanolic extracts from the fruit parts of *S. madagascariensis* was tested on human embryonic kidney (HEK293) and cervical cancer (HeLa) cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] cell proliferation assay. The fruit pulp's moisture (6%), ash (11%), protein (5%), fat (1%), fibre (12%), and carbohydrate (12%) contents were significantly different ($P < 0.05$) from the compositions of the fruit pulp (5% moisture, 5% ash, 4% protein, 33% fat, 8% fibre and 11% carbohydrate). The minerals analysed were significantly ($P < 0.05$) higher in the fruit pulp compared to the seed coat (testa). The fruit's seed coat and fruit pulp's methanolic extracts did not show any significant toxicity in the HEK293 cell line. The seed coat's methanolic extract exhibited moderate toxicity in the HeLa cell line. The methanolic extracts from both fruit parts showed a dose-dependent effect in HEK293 and HeLa cell lines. The results of the cytotoxicity analysis revealed the safe nature of the fruit pulp while caution needs to be taken when consuming the seed coat. The seed coat contained $1.39 \pm 0.01\%$ phytic acid, but oxalic acid and phytic acid were not detected in the fruit pulp. This study suggests that the fruit parts could serve as a source of potassium, magnesium, and nitrogen, but poor sources of protein, carbohydrates, and fat (pulp).

Key words: Anti-nutrients, Cytotoxicity, Extracts, Fruit, Minerals, Proximate, Pulp, Seed



INTRODUCTION

Fruits play an important role in the human diet [1]. They are known to contain low amounts of protein, fat, sodium, and high amounts of fibre, potassium, and calcium [2] with various health benefits. Previous studies reported that fruits, as well as vegetables, possess a reservoir of bioactive compounds [3, 4], which gives them their medicinal properties. Thus, the analysis of phytochemical compounds present in plants provides information on functional groups that may impart the pharmacological action of plants [5].

Although indigenous fruits are widely consumed for health benefits, there is not enough information on their phytochemical composition [6, 7], nutritive value, antinutrients, and cytotoxicity of the wild non-conventional fruit plants which significantly contribute to the nutrition uptake of the local population in India [8]. Obtaining adequate information on the phytochemical compositions, functional groups, and nutrient status of non-conventional plants may encourage the utilisation of these plants as a source of essential nutrients and increase their acceptability for nutraceutical and pharmaceutical purposes.

Strychnos madagascariensis is a member of the Loganiaceae family [9]. The family consists of trees, shrubs, and herbs with about 200 species [10] native to tropical and subtropical Africa, which includes South Africa. The fruit is commonly found in woodlands, rocky places, riverine fringes, and the coastal forest of southern Africa. Though *S. madagascariensis* is widely distributed in Southern Africa, it is underutilised. The seed pip is inedible, but the seed coat and fruit pulp can be used as food for humans. Because of its flavor and pleasant taste, people in rural communities in Southern Africa consume the fruit, particularly women and children [11]. It is pertinent to mention that this fruit is utilised by the locals in Kwazulu-Natal, South Africa with the claim to curb diabetes and hypertension. The fruit is locally processed into different fruit products such as dried fruit skins, juices, and jams with the potential of generating earnings for indigenes of Southern Africa [11]. However, attention has not been given to its potential commercialisation due to limited information regarding its nutritional composition and health benefits. Hence, this study aimed at determining the nutritional composition and safe nature of the seed coat and fruit pulp of *S. madagascariensis* ripe fruit as a potential nutraceutical.



MATERIALS AND METHODS

Fruit collection and identification

Matured ripe fruits of *S. madagascariensis* (Figure 1) were randomly collected from some trees in August 2019 at Mbazwana (27.4937° S, 32.5882° E) KwaZulu-Natal, South Africa. The fruits were transported to the laboratory on the same day by the University of Zululand's transportation division, authenticated by Dr. N.T. Ntuli at the Department of Botany and the fruit sample (voucher number: VH10) was deposited in the herbarium of the University of Zululand. Given the abundance of a variety of *S. madagascariensis* trees in the chosen location, ripe, healthy fruits, yellowish in colour, were randomly gathered from trees in a single batch to have a full basket.



Figure 1: *Strychnos madagascariensis* trees (A), fruits (B), seeds (C), and pulp (D)

Sample preparation

After the collection of the fruit, the shells were broken using a hard object, and the seeds and pulp were removed separately. New gloves and clean laboratory equipment were used every time to prevent contamination during handling. The seed coats were separated from the hard seed. Both the seed coats and the fruit pulp were sun-dried for a few days to obtain a dry sample. Thereafter, the samples were ground separately and kept in clean airtight containers until required for use.

Proximate analysis

The moisture content of the fruit pulp and seed coat was determined using the oven method by the Association of Official Analytical Chemists (AOAC) [12]. Briefly, the pulverised sample was weighed and heated to about 105°C for 3 hours, cooled in a desiccator, and weighed. The moisture content (%) = (Loss in weight of sample) / (Original weight of sample) x100. The ash content was determined by weighing 2 g of the sample and placed into a muffle furnace of 550 °C until the sample turned into ashes and weighed when cooled [12]. The ash content was calculated as the percentage of the initial weight of the sample. The fat content was determined as a percentage (%) of ether extract using the AOAC method [12]. Briefly, the sample was placed in a fat-free extraction thimble and attached to a reflux condenser. A Soxhlet flask previously weighed was filled with n-hexane (two-thirds) and heated for 6 hours with continuous running water into the reflux condenser to expel hexane vapour. The flask was removed, and allowed to cool in a desiccator and the weight of the flask was recorded. The final weight of the flask was subtracted from the initial weight of the flask and the result represented the amount of oil extracted from the sample. The fibre content was determined through acid (sulphuric acid) and alkali (sodium hydroxide) extractions, using the AOAC method [12]. Boiling sulphuric acid (1.25%; 700 ml) was added to 2 g of the sample and heated for half an hour. The solution was filtered, the entire residue was placed in a beaker with boiling water and sodium hydroxide (1.25%) was added, boiled for half an hour, filtered, and rinsed with boiling sulphuric acid (1%; 25 ml), water (150 ml), and ethanol (25 ml). The filtrate was heated in an ashing dish and recorded after cooling and the residue was burnt for half an hour, cooled, and weight noted. The crude fibre content (%) in each sample was calculated as % crude fibre = (Weight of residue- weight of ash) / (Weight of sample) x100.

The protein content was determined using the Kjeldahl method of nitrogen determination [13]. The sample (2 g) was placed in a digestion tube containing a Kjeldahl digestion catalyst (0.8 g) and concentrated sulphuric acid (15 ml). The tube was placed in a pre-heated digester (420°C) for 30 min in a fume cupboard until a clear mixture was noticed, cooled, diluted with distilled water (50 ml), placed in a micro-Kjeldahl analyser, and dispensed (40% of NaOH; 50 ml) into the sample which has been digested. This was heated for about 4 min to release NH₃ and collected into a conical flask containing boric acid (2%; 25 ml). The ammonia was added to boric acid to yield an ammonium borate solution which was titrated against sulphuric acid (0.1 M) until a purplish-grey end-point was observed. The nitrogen content (%) = 0.28/ (weight of the sample (g)) x A. Where A= Volume (ml) of 0.1 M of H₂SO₄. The carbohydrate content of the fruit pulp and seed coat of



S. madagascariensis was determined following the acid hydrolysis method using a spectrophotometer [14].

Mineral analysis of *S. madagascariensis*

The Calcium, Magnesium, Potassium, Sodium, Zinc, Copper, Manganese, Iron, Phosphorus, and Nitrogen contents were determined using AOAC's method [12]. The sample was transferred into a digestion tube (75 ml). The digestion mixture (5 ml) was added while rotated and placed in a fume cupboard and digestion was carried out at 150°C for several hours. The mixture was removed from the digester, cooled for 10 min, and then hydrochloric acid (6 M; 3 ml) was added to each tube. The mixture was digested for another 1 hr 30 min, removed from the digester, cooled, and stirred vigorously using the vortex mixer after the addition of distilled water (300 ml). The mineral component of each sample (seed coat and fruit pulp) was determined using an Atomic Absorption Spectrophotometer which was standardised by the standard of each mineral component. The Ca, Mg, K, Na, Zn, Cu, Mn, Fe, P, and N contents of the seed coat and fruit pulp were analysed.

Anti-nutritional investigations

Determination of phytic acid

The phytic acid content was determined using the protocol described by Lucas and Markakas [15]. The sample (2 g) was placed in a conical flask (250 ml), soaked for 3 hours using concentrated HCl (2%; 100 ml), and then filtered using filter paper (Whatman). The filtrate (25 ml) was placed in a 250 ml conical flask and ammonium thiocyanate (0.3%; 5 ml) was added and titrated against a standard FeCl₂ solution containing 0.00195 g iron/ml. The formation of a yellow color solution, which continued for about 5 mins was observed and the percentage of phytate was calculated as:

$$\% \text{ Phytic acid} = \text{titer value} \times 0.00195 \times 1.19 \times 100$$

Determination of oxalic acid

Oxalate content was determined using the modified method of Agbaire [16]. The sample (1 g) was placed in a 250 ml conical flask, and 75 ml of 3 M HCl was added. The mixture was stirred with a magnetic stirrer for one hour. The mixture was then filtered, and 25 ml of filtrate was taken and heated to 90°C. The temperature of the filtrate was kept above 70°C. The hot filtrate was titrated against 0.05 M of KMnO₄ until an extremely faint pale pink color was observed within 15-30 sec. The amount of oxalate present was calculated by considering 1ml of 0.05 M KMnO₄ equivalent to 2.2 mg of oxalate.

Cytotoxicity bioassay of the methanolic crude extracts

The cytotoxicity of the methanol crude extracts of *S. madagascariensis* pulp and seed coat was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] cell proliferation assay [17]. The crude extracts were obtained by air-drying and pulverising the fruit parts separately using a laboratory grinder (Clarkson BB25EP). The pulverised samples were used to prepare two extracts by maceration using methanol (200 ml) and placed on an automatic shaker (150 rpm; 25°C) for 72 hr. The extracts were filtered using filter paper 1 (Whatman) and the Heidolph rotary evaporator (45 rpm, 40°C) was used to concentrate the extracts. The crude extracts were tested on the human embryonic kidney (HEK293) and HeLa cell lines. The cells were seeded in a 48-well plate at a density of 2.5×10^4 cells per well. After an overnight incubation (at 37 °C), the cells were further incubated with the crude extracts at varying concentrations (25, 50, 100, 200 µg/ml) in a medium (MEM+Gutamax+antibiotics+10% fetal bovine serum) for two days. Subsequently, MEM+Gutamax+antibiotics+10% fetal bovine serum was removed from the cells. Thereafter, 200 µl each of MTT solution (5 mg/ml PBS) and cell culture medium were added to the respective wells. These cells were further incubated at 37 °C for 4 h, the reaction was stopped by adding dimethyl sulfoxide (100/200/400 µl), then the viability of the cells was determined using a spectrophotometer (Biomate spectrophotometer) at a wavelength of 570 nm. The experiment was done three times and the results were stated as mean \pm SD. The percentage inhibition of cell growth was calculated using the formula:

$$\% \text{ cell death} = [(Ac - As)/Ac \times 100]$$

Where Ac and As represents the absorbance of the control and extract, respectively.

Data Analysis

The samples were triplicated for each parameter during the experiments, and the mean, standard deviation, and statistical differences (Tables 1 and 2) were determined using SPSS. Paired samples t-test was conducted to compare the means of each parameter (moisture, ash, protein, TNC, fibre, fat, Ca, Mg, K, Na, Zn, Cu, Mn, Fe, P, N) of the seed coat and the fruit pulp for statistical differences (p-value). The graphs were produced using GraphPad Prism.

RESULTS AND DISCUSSION

Proximate compositions of *S. madagascariensis*

The fruits' seed coat and pulp had $5\pm 0.13\%$ and $6\pm 0.13\%$ moisture content, respectively. The ash content was higher in the pulp compared to the seed coat (testa). The fat content was higher in the seed coat when compared to the pulp. The protein content of the seed coat and pulp was 4 ± 0.06 and 5 ± 0.10 , respectively. The fibre and carbohydrate contents were higher in the pulp compared to the seed coat (Figure 2).

With the low moisture content, the sun-dried material is expected to have a long shelf life, thereby reducing its susceptibility to damage caused by microbial activities. The sun-dried pulp had little fat content (1%) which was found to be comparable to that found in Baobab pulp (1.42%) [18], but the seed coat contained a substantial amount (33%) which was far over 1-2% considered adequate fat content for dietary human intake [19]. With such high-fat content, it might be possible to extract oil from the seed coat. The low-fat content of the fruit pulp indicates it could form part of the diet plan for those who intend to control their calorie intake.

With regards to protein content, it has been reported that plant foods with a protein content of about 12% are considered good sources of protein [20]. The protein content of the analysed samples (seed coat and pulp) in this study was below 12%, which implies that they are poor sources of protein as has been noted for many other fruits [21]. The ash content was higher in the pulp ($11.0\pm 0.3\%$) when compared to the seed coat ($5.0\pm 0.10\%$). The ash content determines the total amount of minerals in a sample, without accounting for the specific minerals present. The fibre content of the seed coat ($8.0\pm 0.08\%$) and fruit pulp ($12.4\pm 0.5\%$) (Table 4.2) was found to be above the nutritional maximum level of 3.0% [22] implying that the fruit parts of *S. madagascariensis* could serve as good sources of fibre. The consumption of a high-fibre diet is usually encouraged due to its several health benefits such as the reduced risk of developing diabetes, hypertension, and cancer [23]. The total non-structural carbohydrate (TNC) content of the seed coat (11.0 ± 0.03) and fruit pulp ($12.0\pm 0.01\%$) was lower than those found in Dates fruit (75%), Mango (16.9%), and Apple (13.4%) but higher than those found in Papaya (7.2%) and Orange (10.9%) [24].



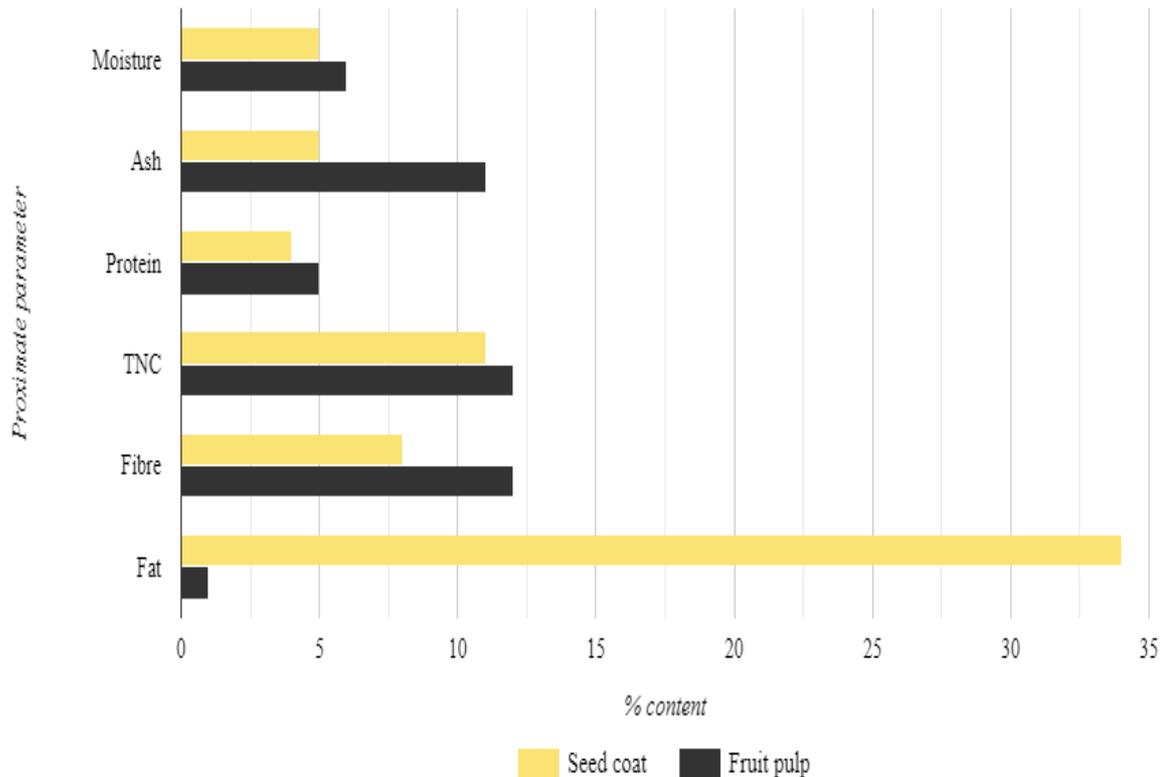


Figure 2: Proximate parameters of the seed coat and fruit pulp of *Strychnos madagascariensis*

Mineral nutrients analysis of *S. madagascariensis* ripe fruit

All the minerals analysed (K, Mg, N, Ca, Na, P, Fe, Zn, Cu) were significantly ($P > 0.05$) higher in the fruit pulp when compared to the seed coat (Table 2 and Figure 3). The fruit parts (seed coat and pulp) had a moderate amount of potassium, magnesium, and nitrogen. The potassium content in both fruit parts (1660 ± 0.28 mg/100g and 2108 ± 3.04 mg/100g) was found to be comparable to the adequate intakes of potassium (2000-3400 mg) [25], suggesting that the fruit parts of *S. madagascariensis* are good sources of dietary potassium. Potassium has a positive effect on the cardiovascular system by maintaining a physiological balance between Na^+ and K^+ levels hence regulating and lowering blood pressure [26]. The calcium level in *S. madagascariensis* seed coat and fruit pulp (30 ± 0.43 and 160 ± 0.32 mg/100g, respectively) are lower than the amount recommended daily (500-1300 mg) [27], which makes the fruit pulp of *S. madagascariensis* a moderate source of calcium needed in various biochemical and physiological processes such as muscle contraction, apoptosis, neuronal function, natural resistance, and activation of enzymes [28]. This study showed a significant amount of magnesium in the seed coat and fruit pulp (80 ± 0.31 and 190 ± 0.32 mg/100g), respectively.

These values, though lower than the recommended dietary amount (6 mg/kg daily) [29], imply that the fruit pulp could serve as a moderate source of magnesium which is crucial for the action of vitamin D and calcium in the body.

The sodium level of the fruit was 50 ± 0.47 and 84.85 ± 0.06 mg/100g for seed coat and fruit pulp, respectively (Table 2), which is below the recommended daily requirement (1.5 g) [30]. It implies that the fruit parts are poor sources of sodium which is characteristic of most fruit and vegetables. The phosphorus content of the seed coat (40 ± 0.32 mg/100g) and the fruit pulp (60 ± 0.76 mg/100g) was lower than the recommended daily intake (700 mg) [31]. Thus, both fruit parts are poor sources of phosphorus. The iron contents in the fruit pulp (4.7 ± 0.05 mg/100g) and seed coat (3.4 ± 0.05 mg/100g) were found to be lower than the recommended dietary allowance (8-18 mg) [32], making the fruit parts inferior as a rich source of iron.

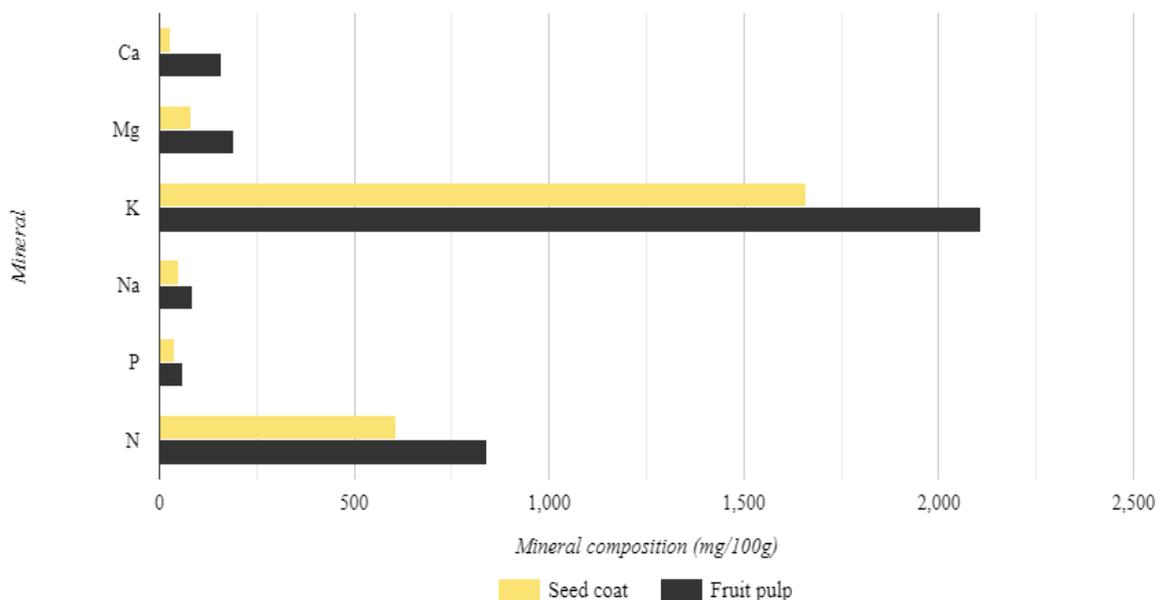


Figure 3: Mineral compositions of the seed coat and fruit pulp of *Strychnos madagascariensis*

Antinutritional compositions of *S. madagascariensis*

Phytic acid was present in the seed coat but lacking in the fruit pulp. Oxalic acid was absent in both fruit parts. Two anti-nutritional compounds commonly present in fruit products are phytic and oxalic acids. Oxalic acid was not found in any of the fruit parts examined. However, low phytic acid content was found; a daily intake above 6% over an extended period can lead to decreased bioavailability of certain minerals in monogastric animals such as rabbits, dogs, horses, and humans [33]. The absence of oxalic acid in the fruit parts is advantageous because one of the

major concerns about oxalate is that it can irritate the mouth and tends to bind a variety of minerals such as calcium and magnesium. Moreover, oxalic acid forms an insoluble complex with iron, thus leading to paralysis and myasthenia [34, 35]. Furthermore, high levels of oxalate in food have been reported to cause excessive urinary excretion of oxalate known as hyperoxaluria. Hence, it increases the likelihood of kidney diseases [36]. This implies that the consumption of this fruit will not interrupt the absorption of essential minerals useful for biochemical reactions in the body system.

Cytotoxicity test

The methanolic extracts of the seed coat and fruit pulp of *S. madagascariensis* ripe fruit showed a dose-dependent effect on both cell lines (Figure 4). The lethal concentration (LC_{50}) of the seed coat and fruit pulp methanolic extracts on HEK293 was 167 and 140 $\mu\text{g/ml}$, respectively whereas, the LC_{50} of the seed coat and fruit pulp methanolic extracts on HeLa (cervical cancer cells) was 39.2 and 194 $\mu\text{g/ml}$, respectively. The pulp and seed coat (testa) showed no significant toxicity on the HEK293 cell line, while the seed coat showed moderate toxicity on the HeLa cell line.

Crude extracts of plants are considered toxic when they inhibit cell viability and growth [37, 38]. Magadula (2014) concluded that the toxicity of a crude extract or pure isolated compound is considered strongly toxic if at a concentration of 10-20 $\mu\text{g/ml}$, moderately toxic at a concentration of 20-100 $\mu\text{g/ml}$, or weakly toxic at concentrations above 100 $\mu\text{g/ml}$ [39]. The methanolic extracts from the seed coat (testa) and pulp of *S. madagascariensis* ripe fruit showed weak toxicity (140-167 $\mu\text{g/ml}$) on the HEK293 cell line, while the methanolic extract from the seed coat showed moderate toxicity (39.2 $\mu\text{g/ml}$) on the HeLa cell line. This confirms that the fruit pulp is safe for consumption while the seed coat needs to be consumed with caution.

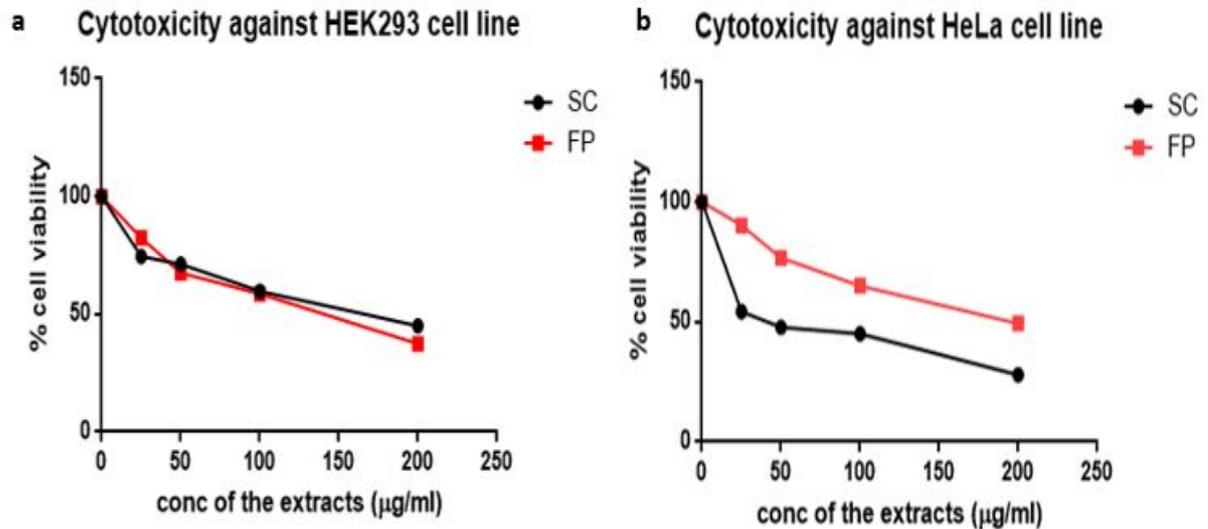


Figure 4: Cytotoxicity of the methanolic crude extracts of *Strychnos madagascariensis* seed coat and fruit pulp on HEK293 (a) and HeLa (b) cell lines

CONCLUSION

This study demonstrated the potential of the seed coat and pulp of *S. madagascariensis* ripe fruit as rich sources of K, and the fruit pulp as a good source of magnesium, nitrogen, and iron. The fruit parts also showed to be a good source of fibre, but a poor source of fat (pulp), protein, and carbohydrate, though the fat content of the seed coat was high. Phytic acid was significantly low in the seed coat implying that the fruit parts (seed coat and pulp) will not prevent the absorption of mineral nutrients in the diet. The cytotoxicity studies provided information regarding the safe use of the fruit pulp while the seed coat should be cautiously utilised.

ACKNOWLEDGEMENTS

The author appreciates the financial support of the Research Office, University of Zululand, South Africa.

CONFLICT OF INTEREST

None declared.



Table 1: Proximate compositions of *Strychnos madagascariensis* ripe fruit

Parameter	Seed coat	Fruit pulp	p-value
Moisture content (%)	5.13±0.32	6.13±0.32	0.003
Ash content (%)	5.00±0.10	11.13±0.32	<0.001
Fat content (%)	33.57±0.35	1.00±0.50	0.001
Protein content (%)	3.98±0.08	5.00±0.10	<0.001
Fibre content (%)	7.90±0.08	12.43±0.50	0.006
TNC content (%)	10.98±0.03	12.01±0.03	<0.001

Values are expressed as mean ±SD

Table 2: Mineral nutrient composition of *Strychnos madagascariensis* ripe fruit

Mineral	Seed coat (mg/100g)	Fruit pulp (mg/100g)	p-value
Ca	29.65±0.52	160.13±0.32	<0.001
Mg	80.15±0.31	190.13±0.32	<0.001
K	1660.17±0.29	2108.50±3.04	<0.001
Na	50.59±0.47	84.86±0.08	<0.001
Zn	0.10±0.01	0.73±0.06	0.003
Cu	0.40±0.01	0.42±0.006	0.130
Mn	2.03±0.23	6.77±0.06	0.001
Fe	3.37±0.06	4.77±0.15	0.002
P	40.13±0.32	59.83±0.76	<0.001
N	608.50±3.04	840.17±0.28	<0.001

Values are expressed as mean ±SD



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