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Chemical and Phytochemical Compositions of *Voandzeia substerranea* Seeds

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Abstract: The seeds of *Voandzeia subterranean* (L.) Thouars (Fabaceae), from Congo-Brazzaville were studied for proximate, qualitative and quantitative compositions. Phytochemical screening of various solvent extracts showed the presence of alkaloids, flavonoids, glycosides, saponins, steroids, triterpenoids, phenols, anhocyanins and carotenoids. Tannins and anthraquinones were not found. Quantitative analysis showed a high amount of alkaloids (34.40±0.2%), flavonoids (4.93±0.17%), saponins (2.20±0.11%) and anthocyanins (1.00±0.12%) in decreasing order. Phenols (0.60±0.12%) and carotenoids had low yields (0.26±0.07%). Proximate analysis of the seeds showed high moisture, carbohydrate and energy content values (49.14, 20.53% and 956.14 kJ 100 g^{-1}, respectively). The results showed low ash content (3.84%) and the relatively high fat (7.84%) and protein content (18.65%). The mineral analysis revealed that potassium (3.15%) and phosphorus (1.74%) were the most abundant minerals. Calcium (0.35) and magnesium (0.39%), were found in low amounts. Sodium, iron and aluminum were detected in trace quantities (0.01%). Manganese was not detected in the present study. The seeds were found to be important both for their nutrients and non-nutrients which determined the medicinal and nutritional value of the plant.

Key words: *Voandzeia subterranea*, composition, proximate, mineral, phytochemicals

INTRODUCTION

In recent years many studies have focused on indigenous plants cultivated as subsistence crops (Karthikeyan *et al*., 2009). Such plants contain chemical bioactive compounds contributing to their medicinal value. (Rabe and van Staden, 2000). They are eaten as component of supporting food, as main dishes or as dessert.

Voandzeia substerranea (L.) Thouars (Fabaceae), named the bambara groundnut, is a leafy vegetable that is widely cultivated throughout sub-tropical Africa, in Madagascar and in the Philippines. The seeds are used in human nutrition and the leaves which are rich in phosphorus, are used for animal food. Both the seeds and the leaves are used in traditional medicine (Nacoulma-Ouedraogo, 1996). In Congo-Brazzaville the seeds are used to treat polymenorrhoea (Adjanohoun, 1988).

The literature review reveals few studies on the chemistry of bambara groundnut seeds. Various studies have reported on the biochemical and the nutritive properties of the seeds of *V. substerranea* (Onimawo *et al*., 1998; Minka and Bruneteau, 2000; Fadahunsi and Sanni, 2010). No literature was found on the chemical or biochemical composition of the seeds from Congo-Brazzaville.

The present study was designed to evaluate the chemical and phytochemical composition of *V. susterranea* seeds in order to establish the nutritional and the medicinal value of these crops.

MATERIALS AND METHODS

Plant materials and collection: The fresh seeds of *V. substerranea* were collected from April-May 2010 at Texaco market, kibeliba-Tsieme, in the North area of Brazzaville (Congo). The seeds were identified and authenticated by Nkouka Saminou from the herbarium of the Vegetal Research Centre (CERVE, ex/OROSTOM-Congo) where voucher specimens had been deposited. The seeds were washed and air-dried at room temperature for three weeks. They were slit open and the seeds extracted from the pulp. The seeds were ground with a mechanical grinder and the powder material was stored under cool and dry conditions. Proximate analysis and mineral composition

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analysis were carried out on the dried powder sample. The defatted powder was used for phytochemical analysis.

Chemical analysis

Proximate analysis: The moisture content was determined by drying at 105°C in an oven, until a constant weight was reached. For total ash determination, the plant samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The Kjeldhahl method was used for crude protein determination. Crude fat content was determined by extraction with hexane, using a soxhlet apparatus. All these determinations were carried out according to AOAC (1990). Carbohydrate content was determined by calculating the difference between the sum of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively (Kilgour, 1987).

Mineral analysis: Mineral analyses were carried out according to Martin-Prevel *et al.* (1984). Elemental analyses were carried out using an atomic absorption spectrophotometer and a flame photometer to determine calcium, sodium, potassium and magnesium content. Aluminum, iron and phosphorus were determined calorimetrically. The concentration of each element in the sample was calculated on a dry matter basis.

Qualitative and quantitative phytochemical evaluation

Preparation of fat free sample: The dried powder from the *V. subterranea* seeds was soaked in hexane for 72 h at room temperature to remove lipids. After filtration the lipid fraction was discarded. The residue was air dried to evaporate the remaining solvent and left in dry conditions for solvent extraction.

Extraction procedure: The fat-free powder was extracted with various solvents (ethanol 90%, water, ethylacetate, methanol and chloroform) using the percolation method. Thus 20 g of the defatted sample was percolated with 150 mL of each solvent at room temperature for 72 h. The mixtures were then filtered. The filtrates were evaporated to dryness in the water bath. The residues then obtained were re-dissolved in their own mother solvents and subjected to phytochemical screening.

Preliminary phytochemical screening: Qualitative analysis of *V. subterranea* seeds was carried out according to the methodology described by Trease and Evans (1989), Sofowora (1993), Harborne (1994, 1998) and Kokate (2001) to determine the presence of alkaloids, anthraquinones, flavonoids, glycosides, saponins, tannins, phenols, anthocyanins and carotenoids.

Quantitative phytochemical analysis: Quantitative phytochemical analysis of the samples was performed in order to ascertain the presence of bioactive compounds by using described methods (Harborne, 1973; Boham and Kocipai, 1994; Obadoni and Ochuko, 2002; Onyeka and Nwambekwe, 2007; Hussain *et al.*, 2011). The phytochemicals determined included alkaloids, flavonoids, saponins, phenols, anthocyanins and carotenoids.

Statistical analysis: All data were expressed as Mean±SD from triplicate (n = 3) determinations.

RESULTS AND DISCUSSION

Proximate composition: The results of the proximate composition (Table 1) showed the high moisture content of *V. subterranea* seeds (49.14%). This value was higher than that reported by Ojimelukwe (1999) for *V. subterranea* (8-10%). The high moisture content of these seeds indicates that they will be susceptible to microbial growth and will not have a long shelf life.

The carbohydrate content (20.53%) recorded in this study was lower than the 58.09 and 66.45% reported for *Cola acuminate* and *Cola nitida,* respectively (Dewole *et al.*, 2013). Dietary carbohydrates supply the bulk of calories and bulk in an average diet, yet they make up less than 1% of total body weight (Ismail *et al.*, 2008). They are known to supply the body with energy. Akinyeye *et al.* (2010) reported the high energy value of 1086 kJ 100 g^{-1} for the *P. mildbraedii* leaves as that calculated for the studied sample. This could be justified by the contribution of the high protein and carbohydrate content.

The results showed the moderately high protein content of 18.65% which favorably compared with the 19.60% of *V. subterranea* seed reported by Fadahunsi and Sanni (2010); however, this value was lower than the (78.75%) recorded by Yagoub and Abdalla (2007). The moderated protein content of the seed revealed that the seeds could be considered as a source of protein for humans with moderate protein needs.

The fat content value was relatively high (7.84%) whereas the total ash content of the seeds was low (3.84%). These values were in line with the fat and the ash content of 6-8% (Ojimelukwe, 1999) and 3.26% (Ogunji *et al.*, 2003), respectively recorded in *V. substerranea* seeds.

Table 1: Proximate composition of *Voandzeia substerranea* seeds

Parameters	Composition (%)
Moisture	49.14
Dry matter	50.86
Total fat	7.84
Crude proteins	18.65
Carbohydrate	20.53
Total ash	3.84
Energy (kJ 100 g^{-1})	956.14

Table 2: Mineral composition of *Voandzeia substerranea* seeds

Elements	Content (DW)
Calcium	0.35
Magnesium	0.39
Potassium	3.15
Sodium	0.01
Manganese	0.00
Iron	0.01
Aluminium	0.01
Phosphorus	1.74

The low ash content of *V. subterranea* seeds indicates the low total level of inorganic components in the sample. The fat content of 7.84% of the seeds indicated that it could be used as a potential source of lipids and also oil-soluble vitamins such as A, D, E and K for humans (Ramirez-Mares *et al*., 2010). It has been reported that a diet providing 1-2% of its caloric energy as fat is sufficient for humans. Excess fat consumption may contribute to certain cardiovascular disorders (Antia *et al*., 2006).

Mineral composition: The mineral composition of *V. substerranea* seeds is shown in Table 2. The results revealed that potassium and phosphorus were the most abundant minerals present in the seeds (3.15 and 1.75%, respectively). Similarly, potassium and phosphorus as the most abundant minerals have been detected in *V. subterranea* seeds in the range of 8.95-45.15 mg 100 g^{-1} (Belewu *et al*., 2008) and 164.73-187.13 mg 100 g^{-1} (Ijarotimi and Esho, 2009), respectively. The high potassium content of the seeds was also reported by Ijarotimi and Esho (2009) (57.61-80.62 mg 100 g^{-}). The results showed the low contents of calcium and magnesium, whereas, Belewu *et al*. (2008) recorded these minerals in relatively high concentrations in *V. subterranea* seeds (1.9-15.5 mg 100 g^{-1} and 8.95-46.10 mg 100 g^{-1}). As observed in the present study, iron and sodium were detected in a very low concentrations (iron 0.17-0.95 mg 100g^{-1}) (Belewu *et al*., 2008); iron: 0.15-0.48 mg 100 g^{-1} and sodium: 0.10-0.30 mg 100 g^{-1} (Ijarotimi and Esho, 2009).

Although the results showed the low level of all the elements recorded (except potassium, 3.15%) they are nutritionally and medicinally important based on their contribution to human physiology and the requirement in health management to avoid metal deficiency (Wardlaw and Smith, 2006). The Na/K ratio of 0.003 of the sample found in the present study was lower when compared with the recommended Na/K ratio of 0.6, suggesting consumption of the seeds could be effective and useful for people with blood pressure problems (Akinyeye *et al*., 2010).

Qualitative phytochemical analysis: The results of solvent extraction (Table 3) showed good yields of solvent extracts of *V. subterranea* seeds (all>1%). The high yield was observed in methanol (4.20%), chloroform (2.85%) and ethyl acetate (2.10%) whereas, in ethanol and water it was found to be low (1.40 and 1.15%). This indicates that methanol, chloroform and ethyl acetate were the best solvents for the seed extraction.

Qualitative analysis of solvent extracts of *V. substerranea* revealed the presence of alkaloids, glycosides, saponins, flavonoids, phenols, anthocyanins and caretonoids. Anthraquinones and tannins were not found. Among the detected phytochemicals, glycosides, phenols and carotenoids were the most common in solvent extracts, followed by alkaloids. Saponins, flavonoids and anthocyanins were less present in solvent extracts. Most of the phytochemicals, were detected in methanol extract, however, they were present in smaller quantities in water and chloroform extracts. These findings might be important for industrial and domestic processing of the seeds.

Quantitative phytochemical analysis: The results of quantitative analysis are presented in Table 4. This analysis showed high amounts of alkaloids (34.40±0.2), flavonoids (4.93±0.17%), saponines (2.20±0.11%) and anthocyanins (1.00±0.12%) while phenols and carotenoids were recorded in very low concentrations (ranging from 0.26±0.07 to 0.60±0.12%).

Further analysis of the present results in comparison with earlier works showed that the flavonoid content was higher than the value of 2.12 mg 100 g^{-1} (Bondre and Nathar, 2011) and 0.69% (Prohp and Onoagbe, 2012) reported for *Semecarpus anacardium* and *Triplochiton scleroxylon*. However, this value was much lower than the 69.80 mg 100 g^{-1} reported for *T. triangulare* leaves (Aja *et al*., 2010).

The saponin concentration of 2.20±0.11% recorded in this study was comparable to the 2.23% for the stem bark of *T. scleroxylon* (Prohp and Onoagbe, 2012). However, this value was higher than the 1.48 mg 100 g^{-1} reported for *T. triangular* leaves (Aja *et al*., 2010).

Table 3: Phytochemical screening of *Voandzeia substerranea* seeds

	Solvent extract				
Phytochemical	Ethanol	Water	Methanol	Chloroform	Ethyl acetate
Alkaloids	+	+	+	-	+
Anthaquinones	-	-	-	-	-
Flavonoids	+	-	+	-	-
Glycosides	+	+	+	+	+
Tannins	-	-	-	-	-
Saponins	+	-	+	-	-
Steroids	-	+	+	+	+
Triterpenoids	-	-	+	+	+
Anthocyanes	+	+	-	-	-
Phenols	+	+	+	+	+
Carotenoids	+	+	+	+	+
Extract yield (%)	1.40	1.15	4.20	2.85	2.10

+: Present, -: Absent

Table 4: Quantitative phytochemical analysis of *Voandzeia susterranea* seeds

Compound	Yield (DW)
Alkaloids	34.40±0.20
Flavonoids	4.93±0.17
Saponins	2.30±0.11
Anthocyanins	1.00±0.12
Phenols	0.60±0.12
Carotenoids	0.26±0.07

Alkaloid content value ranging from 0.42 to 0.52% has been reported for some plant species (Ayeni and Yahaya, 2010) which is much lower than the value recorded in the present results. As observed for the studied sample, a high amount of alkaloids were also reported by Aja *et al.* (2010) for the dry samples of *T. triangular* (55.56 mg 100 g^{-1}) and by Kolapo *et al.* (2009) for stem and root of *P. africana* (102.4 and 73.2 mg 100 g^{-1}), respectively.

The recorded phenolic content of 0.60% was found to be comparable to the 0.84 mg 100 g^{-1} reported for *R. avensis* (Hussain *et al*., 2011) but lower than the amounts of 3.22 and 2.43 mg 100 g^{-1} recorded in the roots and the leaves of *S. anacardium*, respectively (Bondre and Nathar, 2011).

The results showed the anthocyanin content was 1.00±0.12% which fell within the range of 0.05-6.63 mg 100 g^{-1} (DW) reported for all potato genotypes (Truong *et al*., 2012). Similar results with low content of anthocyanins ranging from 0.02 to 0.1 g 100 g^{-1} were reported for green leafy vegetables (Onyeka and Nwambekwe, 2007).

Carotenoids (0.26%) have been recorded in low concentrations in the green leafy vegetables (ranging from 0.02- 0.05 g 100 g^{-1}) (Hussain *et al*., 2011). The low level of anthocyanins and carotenoids recorded in the present study could be linked to the fact that these seeds are non-colored pigment plants (Dewanto *et al*., 2002).

The high amounts of flavonoids, saponins and alkaloids in the seeds imply that their consumption may have biological effects on body health. Although, anthocyanins, phenols and carotenoids were detected in lower concentrations, their presence may have implications and could be favorable at these levels since they act probably in synergy with other secondary metabolites.

The phytochemicals in the plants may help to treat or to protect humans against degenerative and chronic diseases (Mbatchou *et al*., 2010). Bioactive compounds like alkaloids flavonoids, saponins etc., are known for their medicinal properties (Akindele and Adeyemi, 2007; Han *et al*., 2007; Kubmarawa *et al*., 2007). They work to improve the health status for consumers through the reported biological and pharmacological activities. Consumption of the seeds of *V. substerranea* is important in nutrition due to the presence of these secondary metabolites which may act on women's body metabolism.

CONCLUSION

The medicinal potential of *V. substerranea* seeds linked to the presence of minerals, phytochemicals such as flavonoids, phenols and alkaloids and other nutrients, implies that consumption of this plant food may help in health care and may protect humans from various ailments. The presence in the seeds of nutritive and non-nutritive bioactive constituents with a wide range of biological activities contributes to the nutritional and medicinal values of *V. substerranea* seeds.

This study has provided scientific knowledge on the nutritional and medicinal potential of *Voandzea substerranea*. As a good source of nutrients and non-nutrients the crops can be recommended for inclusion in the food system of Congo to improve human health care.

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