

RESEARCH ARTICLE

Analysis of nutrients and compounds potentially reducing risks of overweightness and obesity-related diseases in raw and roasted *Adenanthera pavonina* seeds from Samoa

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ABSTRACT

Recently, Samoans have faced to alarming increase in the prevalence of obesity related diseases, which are connected to the consumption of imported calorie-rich and nutrient-poor food products. It is believed that the re-introduction of native diet may mitigate these negative trends. In Samoa, the seeds of *Adenanthera pavonina*, an underutilized leguminous tree, are popularly eaten when roasted. Although the seeds are also used in traditional medicine to treat inflammatory and cardiovascular diseases, information on their nutrients and any compounds potentially reducing risks of related health disorders is very limited. Therefore, the objective of this study was to analyse the nutrients and compounds potentially reducing risks of overweight- and obesity-related health problems in raw and roasted *A. pavonina* seeds from Samoa. The standard analytical and microbiological methods, namely gas chromatography–mass spectrometry, inductively coupled plasma optical emission spectrometry, high-performance liquid chromatography with fluorescence detection, ultrahigh-performance liquid chromatography-tandem mass spectrometry, and Czech technical standard methods, were used for the determination of fatty acids, minerals, tocopherols, phenolic compounds, and B vitamins, respectively. The analyses showed that the lignoceric (17.59% and 18.24%), linoleic (39.80% and 37.88%), and oleic acids (14.67% and 14.75%) were the most abundant in the oil of raw and roasted seeds, with the unsaturated forms present in higher amounts than saturated. The seeds were found to be rich of vitamin E (33.09 and 15.94 mg/100 g), whereas the contents of vitamins B₁, B₂, B₃, and B₆ were rather low. Calcium, magnesium, phosphorus, potassium, and sulphur were the minerals found in the highest concentrations. Salicylic acid (201.01 and 151.95 µg/100 g) has been detected in higher amounts than other phenolic compounds. In summary, the findings of this study indicate that the both raw and roasted seeds of *A. pavonina* are good sources of various health-beneficial nutrients, including those reducing the negative effects of obesity.

Keywords: Chemical composition; Nutritional value; Red bead tree; Underutilized legume

INTRODUCTION

The high percentage of chronic health conditions like diabetes, cardiovascular and degenerative joint diseases has recently been recorded among the populations of both developed and developing countries as a result of the dramatic increase of overweightness and obese incidence

worldwide (King et al., 2013; Yatsuya et al., 2014). For example, in Samoa, the prevalence levels of raised blood pressure and overweightness are reaching 40.0% and 84.6%, respectively (WHO, 2017). In a number of studies, the link between obesity and weight gain to deficiencies in micronutrients (e.g. vitamins and minerals) has previously been described (Garcia et al., 2009). These deficiencies

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are involved in obesity-induced metabolic alterations such as insulin resistance, inflammation and oxidative stress. Consequently, the risk of type 2 diabetes, cardiovascular, inflammatory and neurodegenerative diseases is increased (Roussel, 2017). In reaction to these findings, various functional foods and dietary supplements containing specific micronutrients have been developed from plant materials for people with special nutritional needs, such as chronically overweight individuals with obesity-related metabolic alterations (Baboota et al., 2013). It has analytically been proven that underutilized crop species, including lesser known legumes, are promising materials for the development of such products since they may contain high amounts of specific health-beneficial constituents (Leuner et al., 2013; Huml et al., 2016). However, detailed analysis of the chemical composition of these less-researched crop species is necessary for verification of their nutritional quality and safety (Halimi et al., 2019).

A typical example of one such underutilised crop is *Adenanthera pavonina* L., a legume known as red bead tree, which is naturalized and cultivated throughout the tropics as a food, fuelwood, medicinal, ornamental, shade and timber plant. In several regions of Southeast Asia and the South Pacific, its seeds are popularly eaten roasted or cooked. Various seed preparations are used in traditional medicine to treat inflammatory and cardiovascular diseases (Lim, 2012). In Samoa, the roasted seeds, called “lopa”, are frequently sold in small plastic bags in local market places or roadside stands and eaten by local people similarly to peanuts (Whistler, 2000). The previous nutritional and phytochemical investigations of *A. pavonina* seeds revealed appreciable contents of proteins, polysaccharides, crude oil, minerals, and vitamins (Cerqueira et al., 2011; Chourasia and Rao, 2006; Ezeagu et al., 2004; Sultana and Gulzar, 2012). Several classes of biologically active secondary metabolites (e.g. flavonoids) have also been identified (Yadava and Vishwakarma, 2013). It has recently been shown that the processing of *A. pavonina* significantly affects the nutritional and anti-nutritional composition of seeds, and their milk extract contains higher amounts of certain minerals and vitamins compared to that from soybeans (Nwafor et al., 2017; Afolabi et al., 2018). In addition, the enzymatic treatment of seeds increased their antioxidant activity due to the higher content of phenolic compounds, mainly phenolic acids (Araujo et al., 2019).

As far as previous studies investigating *A. pavonina* effects on obesity-related disorders are concerned, the seed extracts reduced the development of diabetic nephropathy and showed anti-inflammatory and blood pressure-lowering effects in animal models (Adedapo et al., 2009; Koodalingam et al., 2015; Pandhare and Sangameswaran, 2012). In a recent study, galactomannans from seeds

decreased glycaemia, total cholesterol and triacylglycerol in streptozotocin-induced diabetic mice (Vieira et al., 2018). Despite the above-mentioned studies, the nutritional potential of *A. pavonina* for the management of obesity, overweightness and related chronic diseases is still not fully known. Therefore, we decided to perform a detailed analysis of specific nutrients and compounds such as fatty acids, minerals, phenolic compounds, tocopherols and B vitamins in raw and roasted seeds of this species with aimed to evaluate their nutritional potential in reducing the risks of overweightness and obesity-related diseases in Samoa.

MATERIALS AND METHODS

Plant materials

The seeds of *A. pavonina* were collected from a wild population of trees growing close to the village Falealupo (Vaisigano district) on the island of Savai'i (in The Independent State of Samoa). During randomized collection, 1600 fruits (400 g) were gathered from four different trees (400 fruits from each tree). The first half of the sample was used for the analysis of raw material and the second half was roasted. Professor Kokoska authenticated the plant species and a voucher specimen (No. 03014KBFR) is deposited in the herbarium of the Department of Botany and Plant Physiology of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague, Czech Republic.

Samples preparation and dry matter determination

The seeds (200 g) were traditionally roasted over an open fire on a metal sheet, in a manner similar to what is commonly done with peanuts, at approximately 180 °C for 30 minutes. A photograph of raw and roasted seeds is shown in Fig. 1. The edible part of the seed (without the hard shiny red testa) was lyophilized using the FreeZone 1 litter benchtop freeze dry system (Labconco, Kansas City, USA), crushed (IKA A11 analytical mill, IKA-Werke GmbH & Co. KG, Staufen, Germany) and stored under -20 °C conditions until analysis. The residual



Fig 1. Raw (A) and roasted (B) seeds of *Adenanthera pavonina* (measure in cm).

moisture was determined gravimetrically at 130 °C for 1 h via a Scaltec SMO 01 analyser (Scaltec Instruments, Gottingen, Germany) according to the Official Methods of Analysis of AOAC INTERNATIONAL (2012).

Fatty acids analysis

Oil was obtained from 7 grams of the sample by multiple extraction with 180 mL *n*-hexane:dichlormethan 80:20 v/v (Penta, CZ) in a Soxhlet apparatus for 6 h. The fat was recovered under a vacuum, dried over anhydrous sodium sulphate (Lach-Ner, CZ), and stored in a capped bottle at 4 °C until used for analysis.

Minerals analysis

Determination of minerals was carried out after acid digestion of samples performed according to the method previously described by Vanek et al. (2010). Initially, 0.5 g of the sample was kept overnight at laboratory temperature in closed conditions, but not hermetically sealed, in a Teflon vessel (Saville, USA) with 10 mL of 65% HNO₃ (p.a., Lach-Ner, CZ). Then the Teflon vessel was sealed and the mixture was heated at 120 °C on a hot plate for 2 hours. The digested solution was then quantitatively transferred to a 50 mL volumetric flask and filled up to the mark with deionised water. The solution was filtered through 0.45 µm Nylon disk filters (Cronus, UK) prior to analysis. An inductively coupled plasma optical emission spectrometer (DUO iCap 7000, Thermo Scientific, Waltham, USA) operating at 1.15 kW with respective nebulizer and auxiliary gas flow rates of 0.5 and 1 L/min was used for content determination of the selected elements, which were monitored at the following spectral line positions (wavelength in nm): boron (B, 208.959), calcium (Ca, 317.933), copper (Cu, 224.700), iron (Fe, 238.204), magnesium (Mg, 279.079), manganese (Mn, 257.610), nickel (Ni, 221.647), phosphorus (P, 177.495), potassium (K, 766.490), sodium (Na, 589.592), sulphur (S, 180.731), and zinc (Zn, 202.548). Quality of digestion and analysis were controlled using blanks and the standard reference materials (NIST SRM 1575a Pine Needles and NCS DC 73351 Tea).

Phenolic compounds analysis

Initially, the plant material (2 g) was extracted in a Soxhlet-like fex IKA 50 extractor (IKA-Werke GmbH & Co. KG, Staufen, DE) in 70% ethanol in 1/20 (w/v) proportion during three 7-min cycles at 130 °C followed by cooling to 50 °C. The extracts were evaporated on a rotary evaporator, dissolved in 5 mL of 40% methanol and filtered through a Teflon (PTFE) syringe filter (17 mm × 0.45 µm). Standard stock solutions of naringenin, rutin (Indofine, Hillsborough, USA), apigenin, *p*-coumaric, ferulic, salicylic, sinapic, syringic and vanillic acids (Sigma-Aldrich, Prague, CZ) were prepared in methanol at a concentration of

1 mg/mL and subsequently diluted in a 40% (v/v) methanol/water solution at concentrations ranging from 0.1 to 1000 ng/mL.

Ultra-high performance liquid chromatography (UHPLC) tandem mass spectrometry analysis of phenolic compounds was carried out using an Agilent 1290 Infinity instrument (Agilent Technologies, Santa Clara, USA) equipped with a binary pump (G4220B), an autosampler (G4226A), an autosampler thermostat (G1330B) and a column compartment thermostat (G1316C) and coupled to an Agilent triple quadrupole mass spectrometer (6460A) with a Jet Stream ESI ion source. A Kinetex PFP column (2.6 µm, 100 Å, 150 × 3 mm) from Phenomenex (Torrance, USA) was used for the chromatographic separation of the extracts. Column temperature was set at 35 °C and the injection volume was 3 µL. 10 mM formic acid (eluent A) and HPLC grade methanol (eluent B) purchased from Merck (Darmstadt, Germany) were used as mobile phases for a gradient elution. The linear gradient solvent system started at 40% of eluent B and increased to 100% of eluent B in 10 min. This was maintained for 4 min and in 1 min the conditions were returned to the initial ratio, which was maintained for a further 4 min. The flow rate was set at 0.3 mL/min.

The mass spectrometer apparatus was operating in positive and negative mode during the same analysis. The applied conditions of the Jet Stream Ion Source were: drying gas temperature 290 °C; drying gas flow 4 L/min; sheath gas temperature 380 °C; sheath gas flow 10 L/min; nebulizer pressure 35 psi; capillary voltage was set at 3500 and 5000 V in positive and negative acquisitions, respectively.

The multiple reaction monitoring mode was used for the detection. Except for chlorogenic and *p*-coumaric acids, two of the most abundant transitions per analyte were used. An Agilent Mass Hunter (Agilent Technologies, Santa Clara, USA) was used for data acquisition and the quantification of samples.

Tocopherols and B vitamins determination

Tocopherols (α , β , γ , and δ) were determined by HPLC with fluorescence detection according to the method described in Lachman et al. (2013). At first, 0.5 g of lyophilized sample powder was placed into a 50 mL plastic falcon tube, 10 mL of isopropanol (Lach-ner, Neratovice, CZ) was added, then sonicated for 10 min (PS 04, Notus PowerSonic, Vrable, Slovakia), and consequently centrifuged at 8228 g for 5 min (5810R, Eppendorf, Hamburg, Germany). The supernatant was transferred into a 25 mL volumetric flask. The remaining pellet was reextracted twice with 5 mL of isopropanol. The supernatants were combined and made up to volume with isopropanol. An aliquot was filtered

through a syringe filter (PVDF, 0.45 μm) into an amber vial and analysed.

Analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Dionex, Sunnyvale, USA) with a quaternary pump, refrigerated autosampler, column heater and FLD Ultimate 3000 RS detector under the following conditions: analytical column Develosil 5 μ RP AQUEOUS (250 mm \times 4.6 mm) (Phenomenex, Torrance, USA); precolumn Develosil 5 μ C30-UG 100A (10 mm \times 4 mm) (Phenomenex, Torrance, USA); mobile phase consisted of methanol: water (97:3 v/v), (HPLC grade methanol, Lach-ner, Neratovice, CZ; Milli-Q water, Merck Millipore, Billerica, Massachusetts, USA), isocratic elution; flow rate 1 mL/min; injection volume 10 μL ; column temperature 30 $^{\circ}\text{C}$; time of analysis 30 min; detection FLD (excitation 292 nm, emission 330 nm).

Stock standard solutions of individual tocopherols (Merck, Darmstadt, DE) were prepared at concentrations of 100 $\mu\text{g}/\text{mL}$ in methanol and precise concentration (purity) was determined spectrophotometrically according to CSN EN ISO 9936 (2006). Working standard solutions were made at concentrations of 0.05-10 $\mu\text{g}/\text{mL}$. External standard calibrations based on peak areas were used.

The content of vitamin B₁ (thiamine) was determined by the HPLC method according to the Czech technical standard (CSN EN 14122, 2004). The content of vitamin B₂ (riboflavin) was evaluated by the fluorimetric method after its conversion to lumiflavin according to the Czech technical standard method CSN 560054 (1972). The microbiological determinations of vitamins B₃ (niacin) and B₆ (pyridoxine, pyridoxal and pyridoxamine) was performed according to the Czech technical standards CSN 56 0051 (1987) and CSN EN 14166 (2009), respectively.

Statistics and calculations

All analytical experiments were performed in triplicate and results are expressed as means and standard deviations.

RESULTS AND DISCUSSION

The results of the series of chemical analyses performed with raw and roasted *A. pavonina* seeds from Samoa showed considerable amounts of certain types of fatty acids, minerals, phenolic compounds, and vitamins in their edible parts. The detailed results are presented in the Tables 1-4.

The oil yields (w/w) 34.27 \pm 1.20% and 27.68 \pm 1.00% were slightly higher than had previously been reported for raw (9.78%-26.20%) and roasted (11.70%) *A. pavonina* seeds, respectively (Kasirajan et al., 2014; Sultana and Gulzar, 2012; Nwafor et al., 2017). In comparison with previous

Table 1: Fatty acids composition of *Adenanthera pavonina* seed oil

Fatty acid (FA)	Content (%) ¹	
	Raw	Roasted
Saturated (SFA)		
Hexanoic	tr. ²	tr.
Octanoic	0.01 \pm 0.00	0.01 \pm 0.00
Decanoic	tr.	tr.
Dodecanoic	0.12 \pm 0.01	0.13 \pm 0.01
Tetradecanic	0.15 \pm 0.02	0.15 \pm 0.02
Pentadecanoic	0.02 \pm 0.00	0.02 \pm 0.00
Hexadecanoic	10.74 \pm 0.27	11.02 \pm 0.29
Heptadecanoic	0.06 \pm 0.01	0.06 \pm 0.01
Octadecanoic	3.29 \pm 0.18	3.33 \pm 0.19
Nonadecanoic	0.01 \pm 0.00	0.01 \pm 0.00
Eicosanoic	0.68 \pm 0.05	0.68 \pm 0.05
Heneicosanoic	0.01 \pm 0.00	0.01 \pm 0.00
Docosanoic	1.86 \pm 0.17	1.96 \pm 0.18
Tricosanoic	0.10 \pm 0.03	0.10 \pm 0.03
Tetracosanoic	17.59 \pm 1.41	18.24 \pm 1.54
Pentacosanoic	0.23 \pm 0.06	0.22 \pm 0.06
Hexacosanoic	4.53 \pm 0.54	4.72 \pm 0.59
Heptacosanoic	0.02 \pm 0.00	0.02 \pm 0.00
Octacosanoic	0.07 \pm 0.02	0.06 \pm 0.02
Nonacosanoic	tr.	tr.
Triacosanoic	0.04 \pm 0.00	0.04 \pm 0.00
Hetriacontanoic	tr.	tr.
Dotriacontanoic	0.07 \pm 0.03	0.09 \pm 0.03
Total SFA	39.57	40.86
Unsaturated (UFA)		
(9Z)-Tetradec-9-enoic	tr.	tr.
(7Z)-Hexadec-7-enoic	0.03 \pm 0.00	0.03 \pm 0.00
(9Z)-Hexadec-9-enoic	0.13 \pm 0.01	0.15 \pm 0.01
(11Z)-Hexadec-11-enoic	0.01 \pm 0.00	0.01 \pm 0.00
(9Z)-Heptadec-9-enoic	0.02 \pm 0.00	0.02 \pm 0.00
Octadecenoic– <i>trans</i> isomer	0.22 \pm 0.00	0.29 \pm 0.00
(9Z)-Octadec-9-enoic	14.67 \pm 0.37	14.75 \pm 0.38
(11Z)-Octadec-11-enoic	0.41 \pm 0.05	0.44 \pm 0.05
Octadecenoic– <i>cis, trans</i>	0.07 \pm 0.00	0.07 \pm 0.00
Octadecadienoic – <i>cis, trans</i> isomer	0.07 \pm 0.00	0.11 \pm 0.00
(9Z,12Z)-Octadec-9,12-dienoic	39.80 \pm 0.99	37.88 \pm 0.96
Octadecatrienoic – <i>cis, trans</i> isomer	0.03 \pm 0.00	0.03 \pm 0.00
(9Z,12Z,15Z)-Octadec-9,12,15-trienoic	0.24 \pm 0.03	0.21 \pm 0.03
(11Z)-Eicos-11-enoic	2.19 \pm 0.20	2.19 \pm 0.20
(8Z,14Z)-Eicos-8,14-dienoic	0.01 \pm 0.00	0.01 \pm 0.00
(11Z,14Z)-Eicos-11,14-dienoic	0.33 \pm 0.05	0.35 \pm 0.05
(13Z)-Docos-13-enoic	0.31 \pm 0.05	0.31 \pm 0.05
(15Z)-Tetracos-15-enoic	0.57 \pm 0.09	0.50 \pm 0.08
Total UFA	59.08	57.33

¹Mean \pm standard deviation (n=3), ²Traces (below 0.01%)

reports on the chemical composition of the crude oil of *A. pavonina* seeds describing the total content of 21 various fatty acids (Balogun and Fetuga, 1985; Ezeagu et al., 2004;

Table 2: Minerals composition of *Adenanthera pavonina* seeds

Mineral	Content (mg/100 g of dry matter) ¹	
	Raw	Roasted
Boron	1.81±1.13	2.02±1.09
Calcium	455.04±3.96	611.61±2.23
Copper	0.88±0.49	1.81±1.47
Iron	2.80±0.19	4.27±1.41
Magnesium	398.87±7.70	367.43±5.05
Manganese	1.73±0.04	1.50±0.02
Nickel	0.23±0.01	1.66±0.03
Phosphorus	300.89±6.80	319.33±4.31
Potassium	755.94±15.50	754.23±13.70
Sodium	3.60±2.33	3.62±2.13
Sulphur	224.62±4.51	231.96±2.22
Zinc	4.31±0.31	4.68±1.36

¹Mean ± standard deviation (n=3)**Table 3: Phenolic compounds composition of *Adenanthera pavonina* seeds**

Phenolic compound	Content (µg/100 g of dry matter) ¹	
	Raw	Roasted
Flavonoids		
Apigenin	- ²	0.09±0.00
Naringenin	0.03±0.00	0.04±0.00
Rutin	1.08±0.02	0.99±0.01
Phenolic acids		
<i>p</i> -Coumaric acid	2.50±0.02	38.72±0.21
Ferulic acid	16.60±0.11	76.23±0.29
Salicylic acid	201.01±1.21	151.95±3.33
Sinapinic acid	4.75±0.05	3.15±0.02
Syringic acid	0.48±0.00	1.71±0.03
Vanillic acid	6.73±0.13	1.45±0.01

¹Mean±standard deviation (n=3), ²Not detected**Table 4: Tocopherols and B vitamins composition of *Adenanthera pavonina* seeds**

Tocopherol, vitamin	Content (mg/100 g of dry matter) ¹	
	Raw	Roasted
Niacin	1.62±0.09	1.48±0.14
Pyridoxine, pyridoxal and pyridoxamine	0.40±0.02	0.36±0.00
Riboflavin	0.34±0.00	0.31±0.02
Thiamine	0.68±0.01	0.06±0.00
α-Tocopherol	33.09±0.41	15.94±0.64
β-Tocopherol	1.15±0.05	0.70±0.00
γ-Tocopherol	0.87±0.03	0.72±0.02
δ-Tocopherol	0.11±0.00	0.08±0.01

¹Mean±standard deviation (n=3)

Kabele-Ngiefu et al., 1975; Kasirajan et al., 2014; Zarnowski et al., 2004), our detailed chemical analysis revealed the presence of 41 fatty acids, which are listed and quantified in terms of their relative percentages in Table 1.

It was found that (9*Z*,12*Z*)-octadec-9,12-dienoic (syn. linoleic) acid was the most abundant in both raw and roasted samples of the *A. pavonina* seed oil. This is in

correspondence with previous reports of Balogun and Fetuga (1985), Ezeagu et al. (2004), Kasirajan et al. (2014), Sultana and Gulzar (2012), and Zarnowski et al. (2004), however, the content detected in both our samples is slightly lower than the numbers shown in the literature (42.97%-53.5%). In our study, tetracosanoic (lignoceric) acid was the second most predominant constituent of the oil, which agrees with the respective contents 17.5% and 20.24% reported by Kabele-Ngiefu et al. (1975) and Sultana and Gulzar (2012), whilst other authors (Ezeagu et al., 2004; Kasirajan et al., 2014; Zarnowski et al., 2004) have detected significantly lower amounts (3.4%-13.5%). The plasma level of lignoceric acid, a compound usually found in most fats in small amounts (Brielmann et al., 2006), has been found to be negatively related to cardioembolic stroke in a Korean population (Chung et al., 2015). The content of the third most abundant constituent, (9*Z*)-octadec-9-enoic (oleic) acid, was slightly lower than has previously been reported by Balogun and Fetuga (1985), Ezeagu et al. (2004), Kabele-Ngiefu et al. (1975), Karajan et al. (2014), Sultana and Gulzar (2012), and Zarnowski et al. (2004) (15.1%-24.3%). Hexadecanoic (palmitic) and octadecanoic (stearic) acids has been detected in amounts corresponding well with data in the literature ranging from 6.9% to 11.8% and from 1.9% to 4.2%, respectively (Balogun and Fetuga, 1985; Ezeagu et al., 2004; Kabele-Ngiefu et al., 1975; Kasirajan et al., 2014; Sultana and Gulzar, 2012; Zarnowski et al., 2004). The contents of docosanoic (behenic), hexacosanoic (cerotic), and (11*Z*)-eicos-11-enoic (gondoic) acids were below 5%, whereas all other components were present at amounts lower than 1%. It is worth mentioning that in comparison with common edible plant oils, a relatively high content of saturated fatty acids with a higher number of carbon atoms in the molecule was detected in both *A. pavonina* samples. Besides the already mentioned lignoceric acid, cerotic acid was present in unusually high amounts (more than 4.5%). In addition, the significant incidence of a homologous series of higher n-9 acids such as gondoic and (15*Z*)-tetracos-15-enoic (nervonic) acids, was also determined.

Of the total fatty acid profile, it was observed that unsaturated fatty acids (UFA) were present in 1.5- and 1.4-times higher amounts than the saturated fatty acids found in both the raw and roasted samples, respectively, with the profile of UFA predominated by polyunsaturated acids (40.38% in raw and 38.45% in roasted seeds) as against the monounsaturated forms which were detected in both samples up to max. 18.5%. Our results suggest that *A. pavonina* seed oil seems to be a good source of food unsaturated fatty acids that are known to reduce the risk of cardiovascular diseases (Calabrese and Riccardi, 2019).

The results of the mineral content analysis of *A. pavonina* seeds showed that the elements found in higher

concentrations were Ca, K, Mg, P and S, while B, Cu, Fe, Mn, Na, Ni and Zn appeared in much lower amounts (Table 2). K was the most abundant in both raw and roasted samples at levels higher than 750 mg/100 g, whereas the content of Na was very low (~3.6 mg/100 g) – it being generally recommendable for people suffering from hypertension (Perez and Chang, 2014). However, these numbers significantly differ from those previously reported by Ezeagu et al. (2004) for raw seeds (K = 1252.85 and Na = 512.53 mg/100 g) and by Nwafor et al. (2017) for raw and roasted seeds (K = 0.33 and 0.24 mg/100 g, respectively) obtained from Nigeria, which may be caused by differences in the geographical origin of the plant materials. The determined contents of the second most abundant mineral Ca in raw and roasted samples indicate that according to the Recommended Dietary Allowances (RDA), 100 g dry matter of the edible part represents more than 50% of the daily dietary intake of Ca sufficient for adult individuals (800-1200 mg/day) (NRC, 1989). Besides its essential role in osteoporosis therapy, Ca supplementation has been shown to reduce incidence of hypertension and obesity (Coates et al., 2010). The determined contents of Mg in both raw and roasted seeds suggest that 100 g of dry matter of the seed edible part represents almost a whole daily dietary intake of Mg as recommended for adult individuals (270-400 mg/day) (NRC, 1989). Low Mg intake can increase the risk of various cardiovascular and metabolic conditions, including high blood pressure and diabetes mellitus (Coates et al., 2010). The analysed content of P was more than 300 mg/100 g, which indicate that 100 g of dry matter of the seed edible part represents more than one-fourth of its daily dietary intake recommended for adult individuals (800-1,200 mg/day) (NRC, 1989). Since the plasma P status was reported to be inversely related to body weight, its adequate intake is potentially protective against obesity (Obeid, 2013).

It should be noted that the concentrations of Ca, Mg and P significantly differ from numbers reported by Nwafor et al. (2017), who determined 25.61 and 30.34 mg/100 g of Ca, 18.97 and 22.76 mg/100 g of Mg and 7.00 and 6.40 mg/100 g of P in raw and roasted seeds from Nigeria, respectively. Similarly as in the case of K, these differences may be caused by the various geographical origins of the plant materials. Relatively high contents of S were determined in both raw and roasted seeds. It has been proposed that S-containing compounds have clinical applications in the treatment of certain obesity related conditions, such as diabetes (Tappia, et al., 2018). The minority minerals were present in a range from 0.23 to 4.68 mg/100 g. Some of them (Cu, Mn, and Zn) were previously detected in raw *A. pavonina* seeds at similar levels, and only the amount of Fe differs slightly from literature data (Ezeagu et al., 2004; Nwafor et al., 2017). To our best

knowledge, the contents of S, B, and Ni were determined for first time in this study.

The analysis of phenols showed that phenolic acids (coumaric, ferulic, salicylic, sinapinic, syringic, and vanillic acid), flavonoids (naringenin) and flavonoid glycoside (rutin) were found in both raw and roasted seeds (Table 3), while salicylate was detected in the highest amounts. It has previously been proved that dietary salicylic acid, found in varying quantities in fruits, vegetables, herbs, and spices, may reduce the risk of developing cardiovascular diseases and colorectal cancer, both of these pathologies having an inflammatory component (Rinelli et al., 2012). Since the content of this phenolic compound determined in *A. pavonina* is rather higher in comparison with the amounts present in other plant foods such as fruits and vegetables (Wood et al., 2011), it is possible to suppose that regular consumption of the seeds can contribute to their previously described anti-inflammatory action and supports their uses in traditional medicine for the treatment of inflammation (Lim, 2012). Content figures for other the phenolic compounds analysed are significantly lower, not exceeding 80 µg/100 g. Our results describing the presence of flavonoid compounds in *A. pavonina* are in correspondence with the findings of Yadava and Vishwakarma (2013), who identified several flavonoids and flavonoid glycosides in seeds of this species from India. However, the presence of all the above-described phenolic compounds in this plant is reported for the first time in this paper.

As a result of tocopherol and vitamin analyses (Table 4), *A. pavonina* were found to contain all four tocopherol homologues (Fig. 2), whereas α -tocopherol (vitamin E) was found in the highest amounts. A hundred grams of seeds may therefore cover the recommended daily dietary intake of vitamin E for adult individuals (9-12 mg/day) (NRC, 1989), which indicates very good potential for this plant as a source of vitamin E. Besides its well-known antioxidant properties, vitamin E deficiency has been associated with abdominal fat deposition in obese patients (Garcia et al., 2009). The contents of β -, γ -, δ -tocopherols ranged from 0.08 to 1.15 mg/100 g. These results correspond with data of Sultana and Gulzar (2012), who determined a significantly higher content of α -tocopherol than its β -, γ -, δ - homologues in the seed oil.

It has frequently been reported that obesity and overweightness-related diseases can be associated with low levels of vitamins of the group B. For example, vitamin B₁ deficiency has previously been detected in certain populations of people with obesity (Kerns et al., 2015). It has also been observed that vitamin B₂ deficiency can enhance the risk of chronic inflammatory diseases associated with obesity (Mazur-Bialy and Pochech, 2017). In another study, vitamin B₃ reduced the risk of

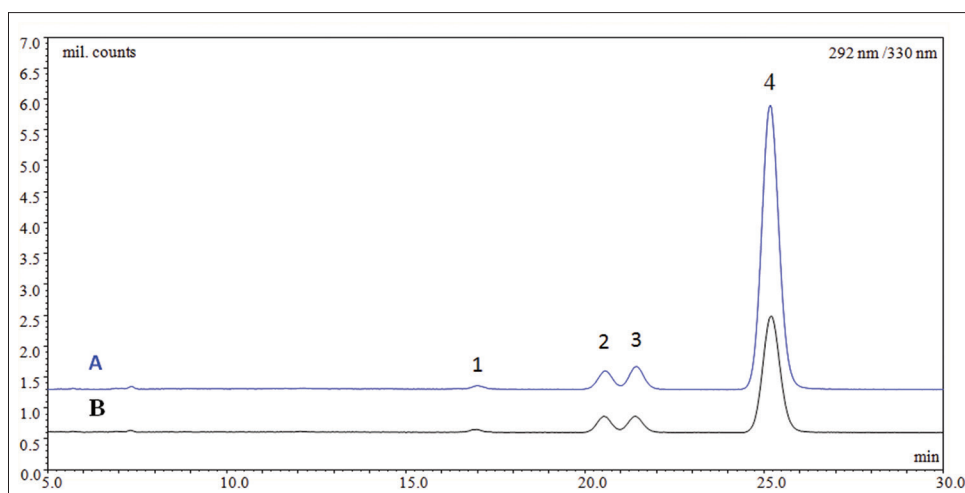


Fig 2. HPLC chromatogram of tocopherols of *Adenanthera pavonina* raw (A) and roasted (B) seeds: (1) δ - (2) γ - (3) β - (4) α -tocopherol

coronary heart disease by decreasing hepatic and plasma concentrations of lipids (Guo et al. 2013). In addition, it has been suggested that the maintenance of an adequate vitamin B₆ status in persons following weight loss diets may help maintain their proportion of fat-free mass (Rodriguez-Rodriguez et al., 2008.). The contents of vitamins B₁, B₂, B₃, and B₆ in raw seeds ranged from 0.06 to 1.62 mg/100 g. To the best of our knowledge, this is the first report on contents of B vitamins in *A. pavonina* seeds.

It well known that process of roasting may lead to significant changes in the chemical composition of legume seeds (Nyembwe et al., 2015). With several exceptions (e.g. vitamin E), the content of substances of traditionally roasted seeds of *A. pavonina* analysed in this study does not substantially vary from those detected in raw seeds. In the case of vitamin E, it is possible to suppose that high temperatures occurring during the dry-heating process may be responsible for its two times lower content in the roasted samples as has previously been reported for dry-roasted peanuts (Eitenmiller et al., 2011). Our results are in correspondence with the study of Nwafor et al. (2017), who reported significant differences in the vitamin E composition of the processed (roasted and boiled) seeds of *A. pavonina* when compared with the raw seeds. However, more detailed research focused on the influence of roasting conditions on the nutrient profile of *A. pavonina* seeds will be necessary for the development of appropriate processing technology.

It is well known that many legumes contain different anti-nutritive and toxic factors, which should be removed/inactivated to improve their nutritional quality and potential for utilization as food or feed (Khazaei et al., 2019). In the case of *A. pavonina*, roasting and cooking are elementary steps in traditional food preparation of seeds because they are believed to be toxic when eaten raw (Lim, 2012). In correspondence with this, the trypsin inhibitor belonging

to the family of Kunitz-type protease inhibitors, which are known to affect the nutritional value of legumes as they inhibit the function of digestive enzymes, such as trypsin and chymotrypsin (Muzquiz et al., 2012), has previously been isolated from *A. pavonina* seeds (Sasaki et al., 2015). Therefore, it is possible to suppose that this heat-labile antinutrient can significantly contribute to the toxicity of raw seeds, whereas thermal treatment would remove its potentially negative effect from consumption as it has previously been reported for soybean trypsin inhibitors (Yang et al., 2014). As far as other potentially antinutritional factors of *A. pavonina* are concerned, we detected (13Z)-docos-13-enoic (erucic) acid in oils from both raw and roasted seeds, however, at very low non-toxic concentrations. Studies done on laboratory animals show that erucic acid appears to have toxic effects on the heart at high doses (Knutzen et al., 2016). Our results correspond well with the previous findings of Sultana and Gulzar (2012) and Kasirajan et al. (2014), who reported content of erucic acid in the range 0.12%-1.6% in raw seeds. Other anti-nutrient constituents such as tannins, phytate, oxalate, and cyanides have also previously been detected in the seeds of *A. pavonina*. There was significant difference in the composition of the roasted seeds when compared with the raw seeds, while the anti-nutrients were generally reduced in the processed seeds (Nwafor et al., 2017).

CONCLUSION

In summary, the results of chemical analysis of raw and roasted *A. pavonina* seeds from Samoa revealed that both of them are promising sources of specific nutrients and compounds, such as fatty acids, minerals, phenols and vitamins, potentially reducing the risk of overweightness and obesity-related diseases. Especially the combination of higher content of UFAs, vitamin E and Ca together with the presence of salicylic acid and a low sodium-

to-potassium ratio suggest the possible positive impact of their regular consumption on cardiovascular system diseases, chronic inflammatory conditions and maintenance of a healthy body weight. In addition, several (micro) nutrients belonging to the classes of minor fatty acids, macro- and micro-elements (S, B, and Ni), flavonoids and phenolic acids (apigenin, naringenin, rutin, coumaric, ferulic, salicylic, sinapinic, syringic, and vanillic acids), and vitamins (B₁₋₃ and B₆), have been determined for first time in this plant. Although this study clearly indicates that the seeds of *A. pavonina* have a promising potential as a novel food source of various nutrients, reducing the risk of health disorders associated with obesity and overweightness, further phytochemical and pharmacological investigations are necessary before its possible recommendation for use in novel food products, for example in form of functional foods and food supplements.

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Authors' contribution

LH and LK: preparation of the manuscript; OD: determination of minerals; BP determination of fatty acids; ZK: determination of tocopherols and B vitamins; MU: sample collection and processing, PM: determination of phenolic compounds, LK: management and coordination of research activities.

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