3 Chemical Composition of *Abrus precatorius* **L. Seeds**

1

4 Abstract

Aims: *A. precatorius* seed powder is traditionally used in Ayurveda, Siddha and Unani
medicine. The objective of present work is to describe the oil, starch, protein,
polyphenol and mineral composition of *A. precatorius* seeds.

8 Methodology: Legumes from *A. precatorius* were collected, and seeds were manually 9 separated. Dried seeds in powder form were employed for the various analyses: solvent 10 extraction was used for elucidation of the oil percentage value; starch content was 11 determined by the enzymatic method; total polyphenol and flavonoid contents were 12 spectrophotometrically analyzed using Folin-Ciocalteu and aluminum chloride as the 13 color developing reagents, respectively; and X-ray fluorescence (XRF) was used for the 14 mineral contents assessment.

Results: The seed kernel consisted of stored oil (3.2%), protein (92.0%) and starch (4.8%). The total polyphenol and flavonoid contents were 24710 and 2520 mg/kg (dw).
A remarkably high content of polyphenols was observed in the seed coat and the seed pod. P, S and (mainly) K nutrients were hyper-accumulated in the seed kernel. The seeds showed a glass transition at -21 °C, two endothermic peaks at 109 °C (dehydration and protein unfolding) and at 209 °C, and a calorific value (~406 kcal/100

g dw) that exceeded those of *Pisum sativum* L., *Lens culinaris* Medik. and other
common pulses.

Conclusions: The seed kernel from *A. precatorius* was mainly composed of stored protein, with low oil and starch contents. High contents of polyphenols, K, Mg, Ca and Fe were found in the seeds. Heavy metals were below the safety limits established for human consumption.

Keywords: *Abrus precatorius* seed, Minerals, Oil, Polyphenols, Protein, Starch,
Thermal properties.

29 1. INTRODUCTION

Plants contain many biological compounds in their bodies [1]. A. precatorius L. is a 30 31 perennial high-climbing, twining woody toxic vine, commonly known as rosary pea, jequirity or Gunja (in Hindi), which can be abundantly found all throughout the plains 32 of India as a weed. The roots, leaves and seeds of this plant of the Fabaceae family have 33 found medicinal uses [2,3], and it has been reported to have antiepileptic, antiviral, 34 antifertility, 35 antimalarial, antidiabetic, neuroprotective, neuromuscular, nephroprotective and immunomodulatory effects, immunostimulatory properties and 36 37 anti-inflammatory activity [4,5]. The seeds are considered abortifacient, aphrodisiac, antimicrobial, diuretic and poisonous due to presence of *abrin*, and have been found to 38 39 be useful in affections of the nervous system and for external use in skin diseases, ulcers 40 and hair affections [3]. Their antinutritional factors (total free phenols, tannins, trypsin 41 inhibitor activity and haemagglutinating activity) have also been investigated [6,7]. The

42 seed proteins are rich in most of the essential amino acids, and are deficient only in 43 cysteine and threonine, when compared to the World Health Organization/Food and 44 Agriculture Organization of the United Nations (WHO/FAO, 2011) requirement 45 pattern.

In this work, the nutritional potential and thermal characteristics of *A. precatorius* seedsare described.

48 2. MATERIAL AND METHODS

49 **2.1. Chemicals and Reagents**

50 AR-grade Folin-Ciocalteu reagent, aluminum chloride, tannic acid, gallic acid and 51 quercetin were supplied by Sigma-Aldrich, and were used for the analysis of the 52 phenols. AR grade sodium maleate buffer, sodium acetate buffer, potassium hydroxide, 53 amyloglucosidase, pancreatic- α -amylase, and glucoseoxidase–peroxidase were 54 purchased from Megazyme International Ireland Ltd., and were used for the starch 55 analysis.

56 **2.2. Sample Collection**

The *A. precatorius* plant was botanically authenticated with the aid of standard monographs [8]. The plant was ripened in the early summer and collected in May 2017 from the area located in Pt. Ravishankar Shukla University, Raipur (21.25°N 81.63°E), India. The plants (0.5 kg) and surface soil (0.5 kg) samples were collected in separate polyethylene bags. They were transported to the laboratory and sundried for one week in a glass room. The seeds from the legume were manually separated. The *A*. *precatorius* fruit parts and soil were further dried in an oven at 50 °C overnight. The
seed pod, seed coat, seed kernel and soil samples were crushed into fine power and
sieved at 0.1 mm mesh size.

66 **2.3. Seeds Drying**

The moisture content of the seeds was determined by drying seeds at 105 °C in an air oven for 6 h prior to the analysis, and mean values are reported. All characterization results were presented on the basis of dry weight (dw).

70 2.4. Weight Measurements

The mass of seeds was weighed by using the Mettler Toledo (Columbus, OH, USA)
electronic balance (AG245). Three seeds were randomly selected for the weighing and
their mean mass was reported.

74 **2.5. Thermal Characterization**

A DSC 204 F1 Phoenix apparatus (Netzsch, Selb, Germany) was used for the differential scanning calorimetry (DSC) characterization. Data collection was conducted in the 25-300 °C range with constant heating rate of 10 °C per min. The analytical parameters were determined using the in-built proprietary software (Proteus, v.7).

79 **2.6. Oil Extraction**

5.0 g of the powdered seeds were agitated in *n*-hexane (25 mL) in a centrifuge at 2500
rpm for 1 min, according to the procedure described by Górnaś et al. [9]. The combined

supernatant liquids were evaporated in a vacuum rotary evaporator at 40 °C until
constant weight was obtained. The oil content was expressed in % (w/w) on a dry
weight (dw) of the seed basis.

85 2.7. Starch and Protein Analyses

The starch content in the seed kernel was determined by the enzymatic method [10]. The protein content was computed 'by difference', i.e., after subtraction of the oil and starch contents [11].

89 **2.8.** Caloric Value

90 The energy content of the seeds was estimated by multiplying the percentages of91 protein, fat and carbohydrate by the factors proposed by Meiners et al. (11).

92 **2.9.** Polyphenol Content

A sample in powder form (100 mg) was dispersed in 5 mL of an acetone and water mixture (70:30, v/v), which was sonicated in an ultra-sonic bath for 20 minutes at 20 °C, according to the procedure reported by Bertaud et al. (12). The total phenolic content (TPC) of each extract was determined as tannic acid by using the Folin-Ciocalteu reagent as tannic acid [13]. The flavonoid content was determined by the aluminium chloride method as quercetin [14].

99 2.10. Mineral Characteristics

A Bruker (Billerica, MA, USA) III Tracer SD portable spectrometer equipped with a
4W rhodium anode and Xflash SDD 2028 channels was used for the X-ray fluorescence

102 (XRF) elemental analysis of the samples. The calibration was carried out by using
103 standard brown and white cowpea (*Vigna unguiculata* (L.) Walp.) seeds and standard
104 soil sample (NCS DC 73382 CRM) [15].

105 **2.11.** Statistics

106 Total polyphenol, flavonoid, resistant starch, soluble starch and oil content analytical

107 variables were analyzed only for the seeds, whereas the contents of Cl, P, S, K, Rb, Mg,

108 Ca, Sr, Mn, Fe, Cu and Zn elements were determined both in the soil and in the seeds.

109 All analyses were carried out in triplicate.

110 3. RESULTS AND DISCUSSION

111 **3.1.** Physical Characteristics of the Seeds

A group of 6 *A. precatorius* seeds were enclosed in the oblong, flat and truncate shaped yellowish colored seed pod (**Figure 1**). The seeds were scarlet colored, with a black spot, and featured a globose shape. A 125 ± 3 mg per seed weight was obtained for the samples under study. The seed coat was found to be relatively thick (representing $29\pm1\%$ of the seed weight), while the kernel fraction was $71\pm2\%$. The average water content of the seeds was found to be $3.2\pm0.1\%$.



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Figure 1. Image of *Abrus precatorius* L. seeds.

120 **3.2. Thermal Characteristics**

The DSC thermogram of the seed kernel (Figure 2) showed a glass transition at -21 °C 121 (onset at -25.6 °C) and two endothermic peaks at 109 °C and 209 °C. The glass 122 transition probably reflects rotational mobility of side chains within seed glasses (β-123 transition or rotation of hydroxyl groups on sugars), although a melting of the stored 124 125 proteins cannot be excluded. The main endotherm at 109 °C corresponds to dehydration and protein denaturation. The second endotherm, with a peak at 209 °C, can be 126 attributed to melting of the carbohydrates and other components [16-18]. The enthalpy 127 of these effects was found to be 174.4 and 36.17 J/g, respectively. 128



- **Figure 2**. DSC thermogram of *A. precatorius* seed kernel.
- 131 **3.3. Caloric Value**

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The energy content of the seeds was found to be *ca*. 406 kcal/ 100 kg (dw), estimated
by multiplying the percentages of protein, fat and carbohydrate by the factors proposed
by Meiners et al. [11].

135 **3.4. Oil, Starch, Protein and Polyphenol Contents**

The seed reserves consist of oil, starch, protein and other constituents, such as trace elements and bioactive compounds. The oil, starch and protein contents in the seed kernel were found to be 2.2 ± 0.1 , 4.8 ± 0.2 and $93.0\pm1.8\%$, respectively. The concentrations of the soluble and resistant fractions of starch were $0.60\pm0.06\%$ and $4.2\pm0.2\%$. Thus, *A. precatorius* seeds would feature a lipid content similar to that of 141 fenugreek seeds, and a carbohydrate content similar to that of fava beans. The TPC and 142 flavonoids content (Fla) in the seed kernel were relatively low: 14200 and 1900 mg/kg, 143 respectively. In contrast, the TPC (Fla) concentration in the seed coat, seed pod and 144 leaves were found to be 24710±290 (2520±51), 3082±62 (3560±66) and 10230±21 145 (8000±155) mg/kg, respectively. These values were higher than those reported by Jain 146 *et al.* [19] for seeds collected in the Mumbai region.

147 **3.5. Mineral Composition**

The concentrations of P, S, K, Rb, Mg, Ca, Sr, Mn, Fe, Cu, Zn, Mo and Pb in the kernel 148 149 of A. precatorius seeds were found to be 2302, 1841, 11132, 4.0, 1046, 975, 1.0, 25, 213, 13, 48, 1.0 and 1.0 mg/kg, respectively. The values for P, K, S, Mg and Ca in the 150 seed kernel may be regarded as high, while those of other micronutrients (Cu, Mn, Zn 151 152 and Fe) were moderate, and those of Rb, Sr, Mo and Pb were low. It is worth noting that the concentration of Mg was higher than that of Ca. The concentrations of Ca, Mn, Fe 153 and Zn were higher than those reported by Pani et al. [20] for seeds from another region 154 in India. 155

156 **3.6. Bioaccumulation Factors**

The soil was brown colored with a pH value of 7.7. The mean concentrations of Cl, P,
S, K, Rb, Mg, Ca, Sr, Mn, Fe, Cu and Zn were found to be 135, 147, 238, 1370, 7.2,
1480, 6330, 49, 1250, 16700, 48 and 24 mg/kg, respectively. Thus, the soil composition
was dominated by K, Mg, Ca, Mn and Fe. Among them, the highest concentration was
detected for Fe.

The bioaccumulation factors (BC), which describe the accumulation and enrichment of an element in the seed kernel with respect to the soil, were found to be for 15.7, 7.7 and 8.1 for P, S and K, respectively, indicating an hyperaccumulation of these three nutrients. The BC values for Rb, Mg, Ca, Sr, Mn, Fe, Cu, Zn, Mo and Pb were 0.6, 0.7, 0.2, 0.02, 0.02, 0.01, 0.3, 0.9, 1 and 0.04, respectively.

167 **4. CONCLUSIONS**

The results of the present study revealed that the major fraction of A. precatorius seed 168 kernel is mostly composed of protein, with low concentrations of oil and starch. P, S 169 and K mineral nutrients were found to be strongly bioaccumulated in the seed, and at 170 least a 1.9-fold molar excess of Mg over Ca was detected in the seed kernel. As regards 171 polyphenol and flavonoid contents, lower concentrations were present in the kernel than 172 in the seed coat and seed pod. The calorific value of A. precatorius seeds exceeds the 173 food energy value of other Fabaceae seeds. The deletereous effects of antinutritional 174 substances may be minimised by cooking, since they are heat labile. 175

176 CONSENT

177 Not applicable.

178 ETHICS APPROVAL

179 Not applicable.

180 CONFLICT OF INTEREST

181 The authors declare no conflict of interest, financial or otherwise.

182 COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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