Chemical Composition of Caesalpinioideae Seeds

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Authors' Contributions

- *Author SC collected, dried, preserved and prepared the plant and soil samples for*
- *the analysis. In addition, the polyphenol, oil and starch contents were analyzed by*
- *him. Author KSP, designed the whole project and written the paper draft. Author,*
- *EKT monitored the mineral content of the seeds and soils by using the XRF*
- *technique with respect to the relevant reference materials. Author, JMG scanned*
- *and interpret the FTIR spectra for the seeds. Author, PMR scanned and interpret the*
- *seed thermal chromatograms, and edited the paper with upgradation. All the*

Authors read and approved the final manuscript.

ABSTRACT

Aims: Caesalpinioideae species have great medicinal and food values. In this work, six Caesalpinioideae species that grow abundantly in central India were selected for chemical investigation: *Delonix regia, Entada gigas, Leucaena leucocephala, Mimosa pudica, Parkia javanica* and *Senna siamea*. The objective of the present work is to describe the phytochemical and mineral composition and the bioaccumulation potentialities of the seeds from aforementioned species.

Methodology: Spectrophotometric, enzymatic and X-ray fluorescence spectrophotometric techniques were used for the quantification of polyphenols, starch and mineral contents, respectively.

Results: The sum of the total concentrations of 17 macro- and micronutrients (P, S, Cl, K, Rb, Mg, Ca, Sr, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo and Pb), oil, protein and total 39 starch in the six seeds were in the 20253-78489 mg/kg, 3.1-30.1%, 52.9-91.5% and 5.4–41.0% range, respectively. The highest concentrations of Fe, oil and phenolics were observed in *M. pudica* seeds. Both thermal and spectral characteristics allowed to differentiate *M. pudica* and *P. javanica* seeds (with the highest caloric contents) from the seeds from the other species.

Conclusion: The selected Caesalpinioideae seeds are potential sources of the nutrients (i.e., P, S, K, Mg, Ca and Fe) and polyphenols, which are needed for biological metabolism and human health. The presence of heavy metals was well below safety limits, enabling their medicinal uses.

Keywords: Caesalpinioideae, starch, polyphenol, mineral, FTIR, thermal analysis.

1. INTRODUCTION

Caesalpinioideae is a subfamily of the Fabaceae family that includes 150 genera and 2500 species, which generally grow in tropical and sub-tropical regions (**1, 2**). The seeds from six Caesalpinioideae species, common in central India, were selected for the study presented herein with a view to their valorization beyond their most frequent uses as sources of wood, of resin and gum, or of medicinal products.

Delonix regia (Bojer) Raf. ('Gulmohar' or 'Flamboyant') is a fast-growing tree that grows in most subtropical and tropical areas of the world and that is harvested for a range of uses, including medicines, timber, fuel and beads (**3, 4, 5**). Its seeds contain gum that is mainly used in the textile and food industries, but which is also being investigated for other applications (e.g., as a binder for the manufacture of tablets) (**6**).

Leucaena leucocephala (Lam.) de Wit ('Subabul' or 'white Popinac') is a perennial small tree mostly cultivated for fodder, as it is an excellent protein source (**7, 8**), but also as a bioenergy crop (**9, 10**). Its dried seeds can be also roasted and used as a coffee substitute due to emollient property.

Entada gigas (L.) Fawc. & Rendle (*Mimosa gigas* L., known as 'sea heart') is a perennial climbing shrub, known to be a rich source of saponins and commonly used for washing hair, clothes etc. (**11**). Its seeds and bark are astringent, and, together with its leaves, they have found numerous applications in Ayurvedic medicine.

Mimosa pudica L. is a creeping perennial herb, usually cultivated as a green manure and for soil stabilization, which is also used in folk medicine (**12, 13**). Its applications as a source of bioactive products for pharmaceutical applications have been reviewed in (**14)**.

Parkia javanica Merr. ('Tree bean' or 'Khorial') is found in most of South East Asian countries. Various parts of the plant are edible, and its bark and pods are used for treatment of various ailments, including intestinal disorders, bleeding piles, diarrhea and dysentery (**15, 16)**.

Senna siamea (Lamarck) H.S. Irwin & Barneby (*Cassia siamea* Lam., 'black wood cassia') is a medium sized tree commonly planted in avenues and gardens, whose leaves can be used as manure and whose flowers are used as a vegetable. *Senna siamea* plays a key role in Jamu (Indonesia traditional medicine), as it possesses many medicinal properties (**17, 18)**. Its chemical constituents and bioactivities have been reviewed in (**19**, **20**).

In this work, the nutritional, phytochemical, spectral and thermal characteristics of seeds from aforementioned six Caesalpinioideae species are described, together with an analysis of bioaccumulation factors and of the correlations found among the various constituents.

2. MATERIALS AND METHODS

2.1. Sample Collection

The seed legumes from plants: *D. regia* (DR)*, L. leucocephala* (LL), *P. javanica* (PJ), *S. siamea* (SS)*, E. gigas* (EG) and *M. pudica* (MP) were collected in April– 91 May 2017 in Raipur area (21° 15′ 0″ N, 81° 37′ 48″ E), after botanical recognition using a standard monograph (**21**). The legumes (pods) were washed out with the de-ionized water and dried with the hot air. The surface layer of the soil on which the plants grew was also sampled. All samples were sundried for one week in a glass

room. Size and mass of the seeds were measured using a Vernier scale and a Mettler-Toledo electronic balance, respectively.

97 Samples were then kept in an oven at 50 $\mathrm{^{0}C}$ overnight for further dehydration, 98 crushed with the help of mortar into fine powder (particle size $\leq 100 \,\mu m$), and stored 99 in glass bottles at -4 ⁰C.

2.2. Characterization

101 The moisture content present in seeds was evaluated by drying the seeds at 105 $\mathrm{^{0}C}$ in an air oven for 6 h prior to the analysis, and mean values were computed. All characterization results were reported on a dry weight (dw) basis.

The infrared spectrum was characterized using a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 Fourier-Transform Infrared (FTIR) spectrometer, equipped with an in-built diamond attenuated total reflection (ATR) system. The spectra were 107 collected in the 400-4000 cm^{-1} spectral range with a 1 cm^{-1} spectral resolution and 64 scans.

Thermogravimetric/derivative thermogravimetric analyses (TG/DTG) and differential scanning calorimetry (DSC) analyses were conducted with a Perkin-Elmer (Waltham, MA, USA) STA6000 simultaneous thermal analyzer by heating 112 the samples in a slow stream of N_2 (20 mL·min⁻¹) from room temperature up to 800 $\,^0C$, with a heating rate of 20 $\,^0C$ ·min⁻¹. Pyris v.11 software was used for data analysis.

AR grade sodium maleate (CAS 371-47-1) buffer, sodium acetate (CAS 127-09-3) buffer, potassium hydroxide (CAS 1310-58-3), amyl glucosidase (CAS 9032-08-0),

pancreatic-α-amylase (MDL MFCD00081319), and glucose oxidase–peroxidase purchased from Megazyme International Ireland Ltd., and were used for color development for spectrophotometric determination. The soluble and resistant starch contents in the seeds were analyzed by the enzymatic method (**22**).

The oil content of the samples was analyzed by equilibrating a 5 g powdered sample with n-hexane (CAS 110-54-3, Sigma Aldrich) as prescribed by *Górnaś* et al. (**23**). The oil fraction was reported as a percentage on the basis of the dry weight (dw) of the seeds.

125 The seed kernel is composed of the oil, protein and starch. In this work, the protein 126 content in studied seeds was computed by subtracting the sum of total concentration of oil and starch to a value of 100 (**24**).

Sigma Aldrich analytical grade Folin-Ciocalteu reagent (MDL MFCD00132625), aluminum chloride (CAS 7446-70-0), tannic acid (CAS 1401-55-4), gallic acid (149-91-7) and quercetin (CAS 117-39-5) were used for the analysis of the phenols. For the determination of total polyphenol content (TPC) and flavonoid content (Fla), 100 mg of sample in powder form was equilibrated with 5 mL of an acetone:water 133 mixture (70:30, v/v), and the solution was sonicated for 20 min at 20 $^{\circ}$ C in an ultrasonic bath, according to the procedure reported by *Bertaud* et al. (**25**). The TPC of each extract was analyzed using Folin-Ciocalteu reagent, and expressed as tannic acid equivalents (TAE) (**26**)*.* The Fla content was determined by the aluminum chloride method, and expressed as quercetin equivalents (QE) (**27**).

A Bruker Tracer 5i portable X-ray fluorescence (pXRF) spectrometer, equipped with a 4W rhodium anode and Xflash Silicon Drift Detector (SSD) with a typical resolution of 2028 channels, was used for the elemental analysis of the seed and soil samples. Two standard reference materials, brown and white cowpea (*Vigna unguiculata* (L.) Walp*.*) seeds, with reference values from ICP-OES and MS (As, 143 Mo and Se in mg kg^{-1}) after *aqua regia* (HCl: HNO₃, 4:1) digestion were used for validation of the pXRF results. A standard soil sample (NCS DC 73382 CRM) was employed for the soil analyses. In soil analytical data, the confidence limit at p value of 0.05 was used.

Bioaccumulation factors were computed by dividing the seed analyte content by the soil one.

2.3. Statistics

Polyphenol, flavonoid, starch and mineral analyses of the seeds were carried out in triplicate. The protein content (%) was determined by subtracting the oil and starch values percentage to a number:100. All values were reported as an average across three replicates with the STD. Correlation coefficients were calculated in IBM SPSS (Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Physical Characteristics of Seeds

The Caesalpinioideae seeds were enclosed in a seed pod. Among those seed pods, that from *E. gigas* was the largest (1-2 m long and 10-12 cm wide). The number of seeds per seed pod were 20–25, 9–12, 15–20, 3–5, 10-15 and 15–20 for DR, EG, LL, MP, PJ and SS, respectively. All Caesalpinioideae seeds studied were brown colored, albeit with different shapes (elliptical*,* ovate or heart shaped, as depicted in **Figure 1**). The mass per seed varied from 21 to 23623 mg (**Table 1**): those from EG were exceptionally large (23623 mg), those from DR and PJ were of moderate size (304–510 mg), and the ones from LL, MP and SS were small (21–61 mg). The moisture content in the six seeds varied from 3.2 to 8.3%, with a fair correlation 166 with mass size $(r = 0.57)$.

Seed coats were found to range from thin to relatively thick: those of the seeds from MP and SS were found to be very thin, while the seed coat of other four seeds (DR, EG, LL and PS) were thicker, contributing from 37 to 69% of the mass of the whole seed. In particular, EG seed coat mass was 9449 mg per seed.

3.2. Polyphenol Content

Total polyphenols and flavonoid contents in the kernels/seeds varied from 1180 to 18840 mg/kg and from 2650 to 9100 mg/kg, respectively (**Table 1**). The highest TPC values corresponded to MG and EG seed kernels. Remarkably high TPC and Fla concentrations were identified in the seed coats, ranging from 26900 to 32000 mg/kg and from 3900 to 12000 mg/kg, respectively.

3.3. Oil, Starch and Protein Content

The phytochemical content in the seeds is shown in **Table 1**. The oil content in the seeds from the six species studied herein varied from 3.1 to 30.1%. Seeds from MP and PJ featured the highest oil contents (17.2 and 30.1%), comparable to those reported for other Caesalpinioideae seeds (**28, 29**).

- Starch contents in the Caesalpinioideae seed kernels were in the 5.4 to 41.0% range.
- The highest starch content was detected in the EG seed kernel, for which the

estimated amount of starch per seed was estimated to be 5811 mg. The content in resistant starch in the seed kernels from the studies species ranged from 0.5 to 1.2%.

186 The protein value was evaluated by subtracting the sum of total value of oil and

187 starch to 100 to express in term of percentage. The concentration of stored protein

was varied from 52.9 to 91.5% with a maximum value for *Senna Siamea* seeds.

The caloric value can be computed by multiplying by 9, 4 and 2 kcal for each gram of oil, protein and carbohydrate (**24**). Thus, the estimated calorie values of the DR, EG, LL, MP, PJ and SS seed kernels would be 365, 319, 388, 518, 442 and 390 kcal/100 g DW, respectively.

3.4. Mineral Content

The quantification of lighter elements i.e. Li, Be, B, etc. with the XRF technique is 195 difficult. The sum of the total concentrations of 17 elements (viz. P. S. Cl, K, Rb, Mg, Ca, Sr, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo and Pb) detected in the DR, EG, LL, MP, PJ and SS seed kernels was found to be 40324, 20253, 43769, 24606, 78489 and 42969 mg/kg of kernel (DW), **Table 2**. The very high mineral content of the PJ 199 seed kernel would be due to its high content in sulphur (5.1%) , due to the presence of thiol compounds in substantial amounts (**30**). Ten nutrients (P, S, K, Rb, Mg, Ca, Mn, Fe, Cu and Zn) were detected in the seed kernels from the six species, at concentrations (in mg/kg) in the following ranges: 2531-7298 (P), 3305–51438 (S), 5334–20198 (K), 4–24 (Rb), 1414–5916 (Mg), 1015-15236 (Ca), 9–233 (Mn), 54– 507 (Fe), 13–29 (Cu) and 10–75 (Zn). Strontium was detected in all seed kernels except for those from EG, at concentrations ranging from 3 to 132 mg/kg. Cl, Cr and Se were only identified in the LL, DR and JS seed kernels, respectively. Mo and Pb

were detected at low levels (1-3 mg/kg) in the seed kernels from LL, EG and SS; and from LL and MP, respectively. The maximum concentration of P, Rb, Ca Sr and Zn; Mn, Fe and Cu; S and Mg; and K were detected in the SS, MP, PR and DR seed kernels, respectively. Relatively low concentrations (11271 mg/kg) of elements (P, S, K, Rb, Mg, Ca, Sr, Mn, Fe, Co, Cu and Zn) were detected in EG seed coat.

3.5. Bioaccumulation

The pH value of the soil solutions was alkaline, ranging from 7.8 to 8.9, with a mean value of 8.2. The surface layer of the soil on which the plants grew was also analyzed by XRF. K, Mg, Ca, Mn and Fe were the main elements observed. Other elements were detected at moderate to low levels. The average concentrations of P, 217 S, Cl, K, Rb, Mg, Ca, Sr, Mn, Fe, Cu and Zn found were found to be 160 ± 10 , 233±18, 135±10, 1387±127, 7±1, 1488±117, 5964±823, 49±4, 1187±94, 15673±1238, 48±2 and 29±2 mg/kg, respectively.

The bioaccumulation factor (BAF) was computed by dividing the elemental concentration in the seed by the soil mean values. The BAC values for P, S, K, Rb, Mg and Ca were in the 16–46, 14–221, 4–15, 0.6–3.4, 1.0–4.0 and 0.2–2.6 range, respectively. A strong bioaccumulation of P, S and K nutrients was observed. In the seeds from three of the species (DR, MP and SS), very high concentrations of P were accumulated, approximately twice those of S. In the other three species (EG, LL and PJ) the reverse trend was observed, with S concentrations approximately 227 twice those of P. In particular, P was found to be strongly hyperaccumulated ($BAF =$ 14-15) in the seeds from DR and LL. Both Mg and Ca were observed to be moderately hyperaccumulated (BAF = 2.3–4.0 and 1.7–2.6) in the seeds from SJ and SS.

3.6. Correlation Coefficients

Correlation coefficients of seed elements are summarized in **Table 3**. The Fla, Mg, S and Se contents showed a good correlation with each other, either due to coordination with phenol groups and/or accumulation of Mg as sulfur and selenium compounds. A good correlation of the oil content with the flavonoid, Mn and Fe content was observed, ascribed to the bond formation with glycerides. Protein had good correlation with elements i.e. P, K, Co and Zn due to bond formation with the amide groups. Rubidium showed a fair correlation with Mo, a co-factor element in its accumulation. Ca had a good correlation with Sr, probably because the latter would be a substituent element in Ca accumulation. Heavy metals (Fe, Cu, Zn, and Pb) showed good correlations with each other.

3.7. Vibrational Characteristics

The ATR-FTIR spectra of the kernel samples are shown in **Figure 2**. The vibrations from the various functional groups in the molecular constituents of the seed kernels from the six Caesalpinioideae have been identified by their position (wavenumber) (**Table 4**). Such assignments, together the analysis of the intensity of the bands at 247 2923 cm⁻¹, 2853 cm⁻¹ and 1744 cm⁻¹, allowed to differentiate the spectra of *Mimosa pudica* and *Parkia javanica* from the rest, and specially from those of *Leucaena leucocephala* and *Senna siamea.*

The band at 1710 cm⁻¹ (conjugated C=O), 1515 cm⁻¹ (aromatic skeletal) and 778 cm⁻ , frequent in the biomass and seed spectra of plants, were missing in the analyzed spectra.

3.8. Thermal Characteristics

TG-DTG shape and DSC thermal effects were analyzed for all the studied samples 255 (Figure 3). A small weight loss was recorded up to $100⁰C$ (first DTG peak), mainly due to the evaporation of a fraction of free water contained in the seed kernel powder. Upon subsequent heating, a multiple DTG feature with peaks between 200 \degree ⁰C and 410 ⁰C was observed, associated with an abrupt pattern of weight loss. 259 Deconvolution of these features allowed to identify three peaks at 210 $^{\circ}$ C, 320 $^{\circ}$ C 260 and 400 $\rm{^0C}$, which can be put in relationship with the final desorption of all bound water, the decomposition of the polysaccharide molecules with formation of low molecular weight volatiles, and the decomposition process of lignin, respectively.

The shape of the TG curves (**Figure 4**) and the temperature for DTG peaks and DSC effects (**Table 5**) evidence notable similitudes in the decomposition rate of the seed kernel samples from *D. regia*, *M. pudica* and *P. javanica*.

4. CONCLUSIONS

The seeds from *M. pudica* and *P. javanica*; *D. regia*, *L. leucocephala* and *S. siamea*; and *E. gigas* were found to be rich in oil, protein and starch, respectively, in good agreement with their vibrational spectra and thermal behavior. A strong bioaccumulation of P, S and K nutrients was observed in all seeds, with particularly high S and K contents in *P. javanica* seeds (51 g/kg), and in *D. regia* and *L.*

leucocephala seeds (20 mg/kg), respectively. *E. gigas* was found to be a starchy seed, whereas *M. pudica* and *P. javanica* would be oily seeds*.* The *M. pudica* and *P. javanica* seeds featured the highest caloric values. Further studies are currently underway to assess the antibacterial, antifungal and anticancer activities of these seeds.

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- Not applicable.

ETHICS APPROVAL

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise

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390 391 **Figure 1**. **Image of seeds: Delonix regia (A), Entada gigas (B), Leucanea lecocephala (C), Mimosa pudica (D), Parkia javanica (E), Senna siamea (F).**

401 **Figure 1. TG (***solid line***), DTG (***dashed line***) and DSC (***dotted line***) curves for the**

- 402 *Caesalpinioideae* **seed samples.**
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Figure 4. Comparison of the TG curves for the different *Caesalpinioideae* **seed samples.**

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Table 1. Physico-chemical characteristics of Caesalpinioideae seeds.

Parameter	Delonix regia	Entada gigas	Leucanea lecocephala	Mimosa pudica	Parkia javanica	Senna siamea
Color	Glossy brown	dark-red brown	Glossy brown	Brown	Brown	Glossy brown
Shape	Oblong	Heart- shaped	Ovate	Oval to orbicular	Elliptic	Flat ovate
Seed mass, mg	510±11	$23623+$	$61 + 2$	21 ± 1	$304+7$	22 ± 1
Seed coat, %	69	40	47		37	$\overline{}$

TPh = Total polyphenol, Fla = Flavonoid

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Table 2. **Mineral characteristics of Caesalpinioideae seeds.**

ND = Not detectable

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Table 3. **Correlation coefficient matrix of seed elements.**

 413 TPh = Total polyphenol, Fla = Flavonoid

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Table 5. DTG peak temperatures for the kernel samples.

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