

**Phenolic and mineral characteristics of seed coats and kernels from 24 species from Raipur area, India****ABSTRACT**

**Aims:** The objective of the present work is the investigation of the physicochemical characteristics of seed coats and kernels from 24 species with medicinal and food applications.

**Methodology:** Seeds from 24 species (2 herbs, 11 vines and 11 trees), belonging to 13 families, were sampled in Raipur (India) in 2017. The collected seeds were dried and weighed, after which seed coats were manually peeled and separately weighed. Phenolic and mineral contents in the seed coats and kernels were analyzed by spectrophotometric and X-ray fluorescence (XRF) techniques, respectively.

**Results:** The seed coat fraction represented from 12% to 95% of the seed mass, depending on the species. The concentrations of total polyphenols, flavonoids and minerals in the seed coats varied from 1800 to 32300 mg/kg, from 1200 to 26900 mg/kg, and from 5876 to 36499 mg/kg, respectively. In the seed kernels, TPh, Fla and minerals ranged from 780 to 31760 mg/kg, from 300 to 31300 mg/kg, and from 12595 to 40810 mg/kg, respectively. P, S, K, Mg, Ca and Fe were found to be the main macro- and micro-elements. Seed coats from Loganiaceae, Phyllanthaceae, Lauraceae and Rutaceae families featured the highest total polyphenol contents, and those from Lauraceae and Rutaceae families showed the highest flavonoid concentrations. The highest total mineral contents corresponded to seed coats from Lauraceae, Rutaceae and Euphorbiaceae families.

24 **Conclusion:** Indian-laurel and curry tree stand out as promising phytochemical and nutrient  
25 sources.

26 **Keywords:** Seed coat, Seed Kernel, Total polyphenol, Flavonoid, Mineral.

## 27 INTRODUCTION

28 The seed coat protects the internal parts of the seed from fungi, bacteria and insects, and prevents  
29 water loss. It is composed of cellulose, fibre, polyphenols, starch, wax, etc. Its outer layer, called  
30 testa, is generally hard and thick, while its inner layer, known as the tegmen, is softer [1].  
31 Enrichment of various compounds (viz. minerals, cellulose, fibre, polyphenols, starch, wax, etc.)  
32 in seed coats have been reported in the literature [2-7]. Among these phytochemicals:  
33 polyphenols have become the subject of increasing research efforts owing to their potential  
34 beneficial effects on human health [8,9].

35 Among the plants found in the Raipur area, Black Siris (*Albizia odoratissima* (L.f.) Benth.),  
36 Malabar spinach (*Basella rubra* L., syn. *Basella alba* L.), wax gourd (*Benincasa hispida*  
37 (Thunb.) Cogn.), squash (*Cucurbita maxima* (Duchesne) Duchesne ex Poir.), watermelon  
38 (*Citrullus lanatus* (Thunb.) var. *lanatus*), Persian melon (*Cucumis melo* var. *cantalupo* Ser.),  
39 Liane Cacorne (*Entada gigas* (L.) Fawc. & Rendle), tree cotton (*Gossypium arboreum* L.),  
40 physic nut (*Jatropha curcas* L.), Persian walnut (*Juglans regia* L.), hyacinth bean (*Lablab*  
41 *purpureus* (L.) Sweet), calabash (*Lagenaria siceraria* Standl.), Chinese-okra (*Luffa acutangula*  
42 Roxb.), sponge gourd (*Luffa aegyptiaca* Mill.), Indian-laurel (*Litsea glutinosa* (Lour.) C.B.Rob.),  
43 Indian-lilac (*Melia azadirachta* L., syn. *Azadirachta indica* A.Juss.), bitter melon (*Momordica*  
44 *charantia* L.), curry tree (*Murraya koenigii* Spreng.), emblic (*Phyllanthus emblica* L.), East  
45 Indian kino (*Pterocarpus marsupium* Roxb.), Indian sandalwood (*Santalum album* L.), Ceylon-  
46 oak (*Schleichera oleosa* (Lour.) Oken), clearing-nut-tree (*Strychnos potatorum* L.f.), and Indian

47 tuliptree (*Thespesia populnea* Sol. ex Corrêa) are widely used as medicine, food and fodder for  
48 animals [10-23].

49 Accumulation of the nutrients and polyphenols in some seed coats have been reported in the  
50 literature [6, 24-28]. In this work, the physical and chemical characteristics of the seed coats and  
51 kernels from aforementioned 24 species (2 herbs, 11 vines and 11 trees) are analyzed, with  
52 emphasis on their polyphenol contents.

### 53 **METHODS AND MATERIALS**

#### 54 **Sample collection and handling**

55 Seeds from the selected twenty-four species were collected in Raipur area (21.25°N 81.63°E),  
56 Chhattisgarh, India, during their maturation period in 2017. The seeds were manually separated  
57 and sun-dried in a glass room for one week, after which they were further dried in a hot air oven  
58 at 50 °C for 24 h. The mass of the seeds was measured using an AG245 (Mettler Toledo,  
59 Columbus, OH, USA) electronic balance. The seed coats were then carefully peeled with the aid  
60 of a surgical blade and their mass was measured. The separated seed coats and kernels were  
61 crushed into a fine powder, and particles of mesh size  $\leq 0.1$  mm were sieved out. The samples  
62 were preserved in a deep freezer at -4 °C until the analyses were conducted.

#### 63 **Analyses**

64 Sigma-Aldrich AR grade reagents were used for the analysis of polyphenols. 0.1 g of powdered  
65 seed coat were extracted with an acetone:water mixture (7:3, v/v), as recommended by Bertaud  
66 et al. [29]. An appropriate fraction was allowed to react with Folin-Ciocalteu reagent for colour  
67 development, and absorbance was measured at  $\lambda=740$  nm with a UV-1800 (Shimadzu, Kyoto,  
68 Japan) UV-Vis spectrophotometer [30]. Three replicates for each solvent extract were performed

69 to determine the total phenolic content (TPh), which was expressed in terms of tannic acid  
70 equivalents by using a standard calibration curve. For flavonoid (Fla) analysis, a fraction of the  
71 extract was reacted with an aluminium chloride solution to develop a yellow coloured complex,  
72 measuring the absorbance at  $\lambda=410$  nm [31]. The Fla concentration was determined with the aid  
73 of a standard quercetin calibration curve and indicated in terms of quercetin equivalents. Three  
74 replicates for each solvent extract were performed, and results are presented as average values  
75 across the three replicates.

76 A III Tracer-SD portable XRF (Bruker, Billerica, MA, USA) spectrophotometer was used for the  
77 quantification of 15 elements: K, Rb, Mg, Ca, Sr, Al, P, S, Cl, Ti, Mn, Fe, Cu, Zn and Pb.  
78 Standard brown and white cowpea (*Vigna unguiculata* (L.) Walp.) seeds were used as reference  
79 material to standardize the analyte concentration [32].

## 80 **Statistical analyses**

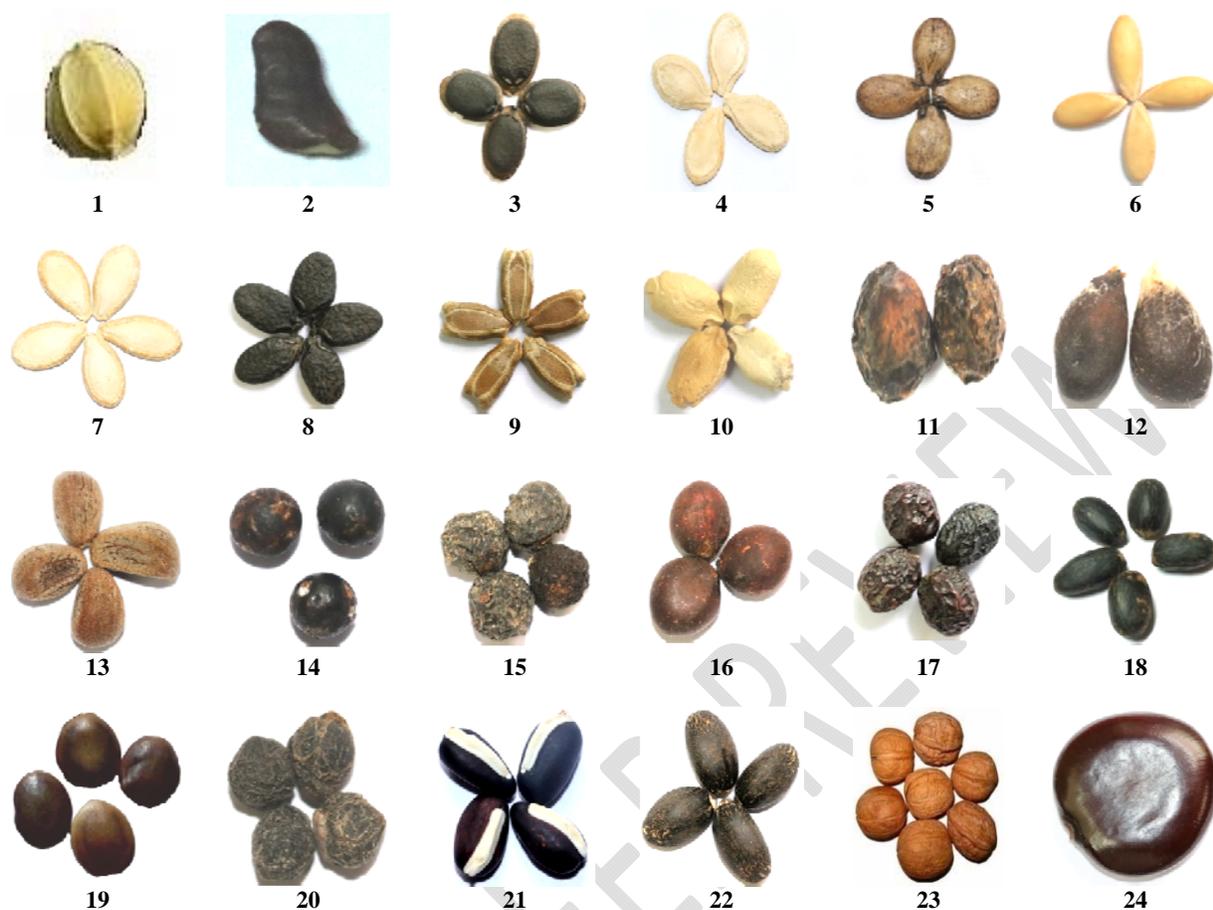
81 Cluster analysis was used to assess similarities in the micro- and macro-elements content in the  
82 seed coats. IBM (Armonk, NY, USA) SPSS v.25 software was used.

## 83 **RESULTS AND DISCUSSION**

### 84 **Physical characteristics**

85 The physical characteristics of the seeds and seed coats under study (shown in **Fig. 1**) are  
86 summarized in **Table 1**. Large differences in seed mass were found, with average values ranging  
87 from 25 to 23623 mg per seed, with the highest weights for the seeds from *Entada gigas* (23623  
88 mg), followed by those from *Juglans regia* (12200 mg). The seed coat mass represented from 12  
89 to 95% of the total seed weight.

90



91 **Fig. 1.** Seed samples from: (1) *P. embilica*, (2) *P. marsupium*, (3) *L. aegyptiaca*, (4) *B. hispida*, (5) *C.*  
 92 *lanatus*, (6) *C. melo*, (7) *C. maxima*, (8) *L. acutangula*, (9) *L. siceraria*, (10) *M. charantia*, (11) *L.*  
 93 *glutinosa*, (12) *G. arboreum*, (13) *T. populnea*, (14) *S. album*, (15) *M. koenigii*, (16) *S. oleosa*, (17) *M.*  
 94 *azadirachta*, (18) *S. potatorum*, (19) *A. odoratissima*, (20) *B. rubra*, (21) *L. purpureus*, (22) *J. curcas*,  
 95 (23) *J. regia*, and (24) *E. gigas*.

96

97 **Table 1.** Physico-chemical characteristics of seeds and seed coats. Total phenolic contents and flavonoid  
 98 contents correspond to the seed coat and kernel samples

Species	Family	Type	Seed mass (mg)	Seed Colour	Seed coat (%)	Seed coat TPh (mg/kg)	Seed coat Fla (mg/kg)	Seed kernel TPh (mg/kg)	Seed kernel Fla (mg/kg)
<i>B. rubra</i>	Basellaceae	V	38	BrB	47±2	11400	10500	3457	1650
<i>C. maxima</i>	Cucurbitaceae	V	132	YeW	18±1	3100	1900	4931	1100
<i>L. siceraria</i>	Cucurbitaceae	V	216	WhBr	42±2	14400	8900	1956	1400
<i>C. lanatus</i>	Cucurbitaceae	V	38	ReBr	49±2	18500	13100	2278	1280
<i>L. aegyptiaca</i>	Cucurbitaceae	V	105	B	43±2	3100	2500	780	620
<i>C. melo</i>	Cucurbitaceae	V	25	LY	28±1	2900	2100	965	300
<i>L. acutangula</i>	Cucurbitaceae	V	122	B	47±2	8300	7200	2144	1380
<i>B. hispida</i>	Cucurbitaceae	V	64	YeW	47±	2900	2600	4074	2280
<i>M. charantia</i>	Cucurbitaceae	V	189	YeBr	35±1	30847	1700	1769	1180
<i>J. curcas</i>	Euphorbiaceae	H	758	Br	47±2	14700	4000	1501	4260
<i>L. purpureus</i>	Fabaceae	V	293	DBr	34±1	22000	3700	1260	2550
<i>A. odoratissima</i>	Fabaceae	T	159	LBr	42±	27000	4100	2492	4300

<i>E. gigas</i>	Fabaceae	V	23623	DBr	40±1	26900	3900	18840	2650
<i>P. marsupium</i>	Fabaceae	T	933	LY	93±3	25800	3800	31760	5800
<i>J. regia</i>	Juglandaceae	T	12200	PY	32±1	9600	1900	1045	1520
<i>L. glutinosa</i>	Lauraceae	T	248	DBr	43±2	29200	26900	4931	3880
<i>S. potatorum</i>	Loganiaceae	T	280	B	24±1	26000	15000	2707	1640
<i>G. arboreum</i>	Malvaceae	H	82	Br	48±2	4000	3500	7263	7160
<i>T. populnea</i>	Malvaceae	T	162	LBr	47±2	16800	8000	15839	12020
<i>M. azadirachta</i>	Meliaceae	T	972	DBr	65±2	1800	1200	1822	1300
<i>P. embilica</i>	Phyllanthaceae	T	920	PW	95±3	27000	3500	4476	3750
<i>M. koenigii</i>	Rutaceae	T	155	B	12±1	32300	25300	3457	3650
<i>S. album</i>	Santalaceae	T	180	DBr	40±2	10900	6200	7075	2750
<i>S. oleosa</i>	Sapindaceae	T	352	DBr	49±2	8500	5400	2198	1950

99 V = Vien, H = Herb, T = Tree, BrB = Brownish black, YeW = Yellowish white, WhBr = Whitish brown, ReBr  
100 Reddish brown, B = Black, YeBr = Yellowish brown, DBr = Dark brown, LuB = Luster black, LY = Light Yellow,  
101 PY = Pale yellow, DB = Dark black, PW = Pale white

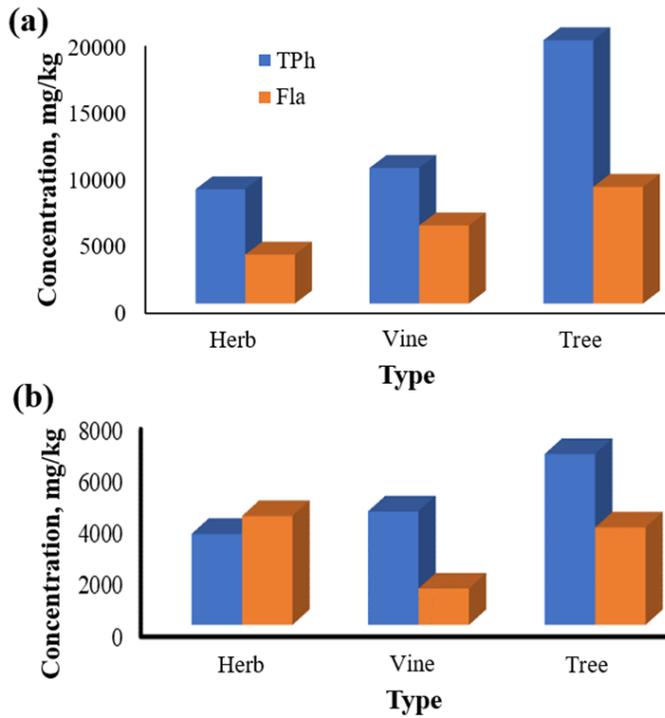
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### 103 Polyphenol contents

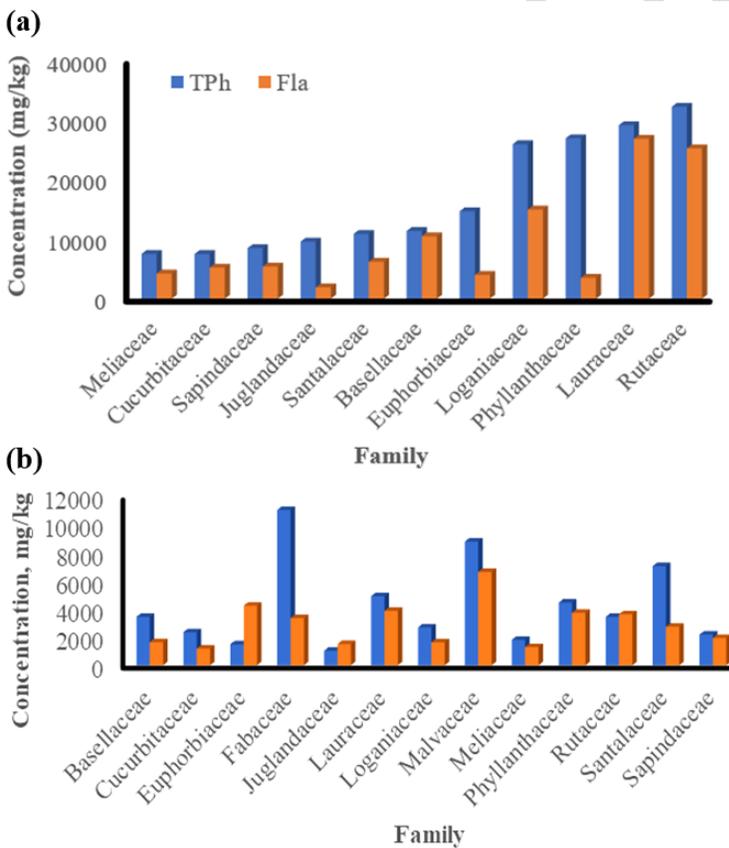
104 The concentration of TPh and Fla in the seed coats and kernels varied from 1800 to 32300  
105 mg/kg, from 780 to 31760 mg/kg, from 1200 to 26900 mg/kg and from 300 to 12020 mg/kg,  
106 respectively (**Table 1**). The [TPh]/[Fla] ratio in the studied seed coats and kernels ranged from  
107 1.1 to 18.1 and 0.4 to 7.1, respectively. Higher contents of TPh and Fla were observed in the seed  
108 coats than in the seed kernels.

109 Large variations in the polyphenol content were observed from one species to another, with  
110 noticeably higher TPh and Fla values in seed coats and kernels from tree species (**Fig. 2**).

111 Similarly, remarkable differences in the polyphenol content of the seed coat and kernel samples  
112 were detected as a function of the family (**Fig. 3**). Loganiaceae, Phyllanthaceae, Lauraceae and  
113 Rutaceae families showed the highest TPh contents in the case of seed coats, while the highest  
114 TPh contents in the case of kernels were exhibited by Fabaceae, Malvaceae and Santalaceae  
115 families. As regards Fla contents, the highest concentrations corresponded to seed coats from  
116 Lauraceae and Rutaceae families.



117  
118 **Fig. 2.** Polyphenol concentration variation in (a) seed coats and (b) seed kernels with respect to plant  
119 types. TPh and Fla stand for total phenolic content and flavonoid content, respectively.  
120



122 **Fig. 3.** Polyphenol concentration variation in (a) seed coats and (b) seed kernels with respect to the  
 123 family. TPh and Fla stand for total phenolic content and flavonoid content, respectively.  
 124

### 125 Mineral contents

126 The mineral contents of 15 elements (viz. K, Rb, Mg, Ca, Sr, Al, P, S, Cl, Ti, Mn, Fe, Cu, Zn and  
 127 Pb) in the seed coats are summarized in **Table 2**. The total concentrations ( $\Sigma_{M15}$ ) ranged from  
 128 5876 to 36499 mg/kg, with the highest values for seed coats from *J. curcas*. Remarkably high  
 129 mineral contents were observed in the seed coats from three families: Lauraceae, Rutaceae and  
 130 Euphorbiaceae (**Fig. 4**).

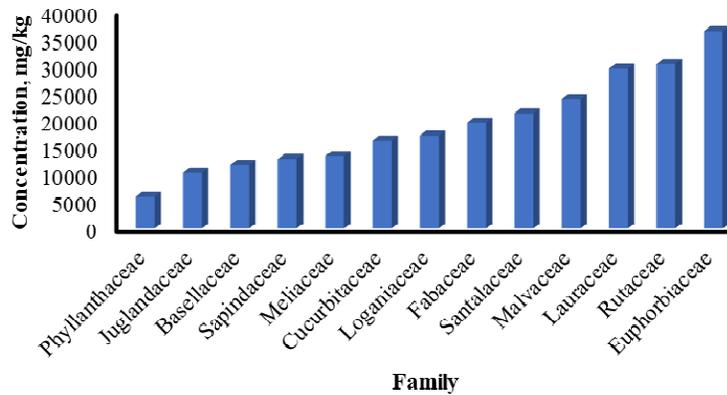
131 P and K nutrients were abundant in the seed coats, ranging from 99 to 4983 mg/kg and from  
 132 1714 to 21982 mg/kg, respectively. The highest P contents were observed in seed coats from  
 133 Cucurbitaceae family, while the highest K contents (>15000 mg/kg) were detected in seed coats  
 134 from *P. marsupium*, *L. glutinosa*, *T. populnea* and *M. koenigii*.

135 **Table 2.** Mineral contents in the seed coats from the 24 species under study, expressed in mg/kg.

Species	Mg	Al	P	S	Cl	K	Ca	Rb	Sr	Ti	Mn	Fe	Cu	Zn	Pb
<i>B. rubra</i>	1012	78	99	194	71	6305	3409	12	4	39	71	389	4	33	3
<i>C. maxima</i>	1762	67	4220	1307	1178	10591	992	13	3	7	85	583	2	4	2
<i>L. siceraria</i>	2020	55	2799	966	88	11324	3833	9	2	9	47	295	2	5	1
<i>C. lanatus</i>	1913	41	3474	1865	78	3247	2965	10	32	8	57	524	11	10	1
<i>L. aegyptiaca</i>	3344	67	2273	909	55	6791	4080	9	1	11	31	142	3	9	1
<i>C. melo</i>	1638	44	4983	1719	101	3913	338	21	1	12	37	364	8	49	1
<i>L. acutangula</i>	1754	81	3486	1302	142	7134	845	9	3	7	20	125	12	10	1
<i>B. hispida</i>	561	98	1878	1063	131	5859	1604	14	3	8	70	313	1	13	2
<i>M. charantia</i>	2642	432	2441	1444	88	5470	2763	8	2	9	77	308	1	3	1
<i>J. curcas</i>	4002	47	1991	1264	91	14636	14210	15	27	11	40	147	13	4	1
<i>L. purpureus</i>	1382	38	2344	1156	48	8541	3176	4	3	7	32	84	6	9	1
<i>A. odoratissima</i>	1738	46	1745	3140	71	8049	6256	15	9	8	132	125	3	5	1
<i>E. gigas</i>	1096	35	104	195	65	6278	3405	16	12	11	58	65	17	27	1
<i>P. marsupium</i>	2098	61	985	1897	59	15236	6685	18	29	38	79	491	914	3	3
<i>J. regia</i>	105	55	254	116	66	7297	2292	3	9	12	17	68	4	4	1
<i>L. glutinosa</i>	542	43	1559	1424	121	21982	3403	23	12	27	94	181	35	27	1
<i>S. potatorum</i>	5808	68	375	975	105	1714	7734	5	2	7	127	166	6	4	1
<i>G. arboreum</i>	2067	77	4001	1399	91	9312	1387	1	5	9	14	137	5.5	40	1
<i>T. populnea</i>	662	87	1631	1062	4859	16894	3256	17	4	11	33	419	402	1	1
<i>M. azadirachta</i>	323	844	826	725	132	7515	2205	9	3	30	28	685	5.5	12	1
<i>P. embilica</i>	175	59	465	243	111	3405	1008	6	20	9	18	348	4	4	1
<i>M. koenigii</i>	1395	49	1311	480	81	20233	6401	7	13	11	21	271	5	11	1
<i>S. album</i>	911	54	886	1100	77	12405	5280	26	29	23	70	327	7	16	1

*S. oleosa* 1635 65 1814 1227 68 2568 4982 3 13 12 34 334 14 6 1

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138

**Fig. 4.** Total mineral contents in seed coats as a function of the family to which the plant species belongs.

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S and Cl concentrations in seed coats were in the 116–3140 mg/kg and in the 48–4859 mg/kg

141

range, respectively. The highest values for S and Cl corresponded to *A. odoratissima* and *T.*

142

*populnea*, respectively.

143

Mg and Ca elements, probably present as silicates, ranged from 105 to 5808 mg/kg and from

144

338 to 14210 mg/kg, respectively. In this case, the highest concentrations of Mg and Ca were

145

observed in seed coats from *S. potatorum* and *J. curcas*.

146

The concentrations of other elements in the seed coats, expressed in mg/kg, were in the

147

following ranges: 1–26 (Rb), 1–32 (Sr), 35–844 (Al), 7–39 (Ti), 14–132 (Mn), 65–685 (Fe), 1–

148

914 (Cu), 1–49 (Zn) and 1–3 (Pb). The highest concentrations of Rb, Sr, Al, Ti, Pb, Mn, Fe, Cu

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and Zn were found in the seed coats from *S. album*, *C. lantus*, *M. azadirachta*, *B. rubra*, *P.*

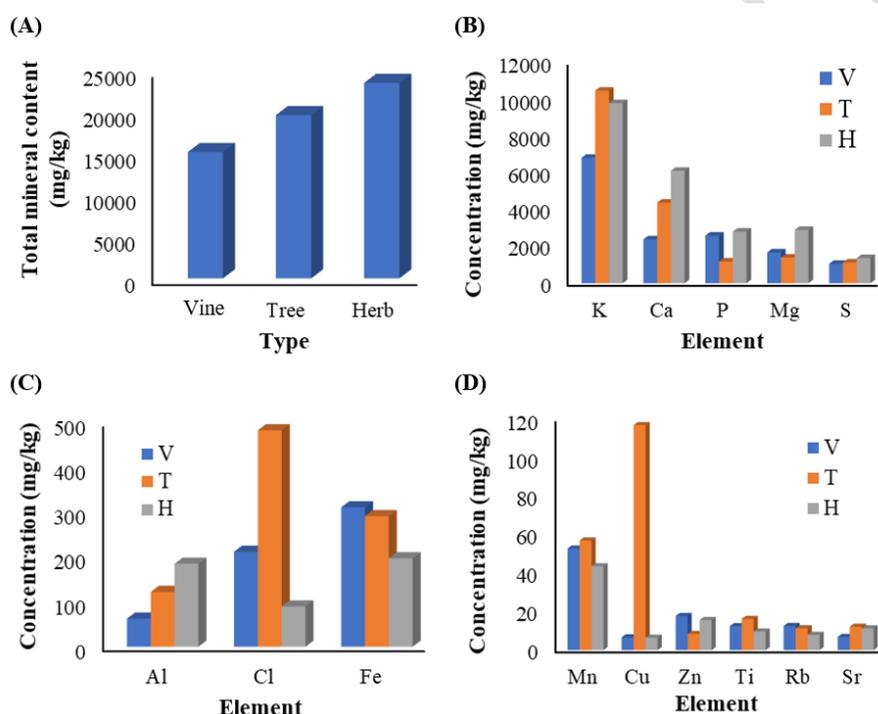
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*marsupium*, *A. odoratissima*, *M. azadirachta*, *P. marsupium* and *C. melo*, respectively. It is

151

worth noting that seed coats from *T. populnea* featured high contents of Cl, K, Fe and Cu.

152 Noticeable differences in the mineral contents were also found depending on plant type. Total  
 153 mineral contents were at least 29 and 55% higher in seed coats from trees and herbs,  
 154 respectively, than in vine samples (**Fig. 5A**). Higher concentrations of major elements (P, S, Mg,  
 155 Ca and Al) were observed in the herb samples (**Fig. 5B**), while those of Cl, K, Mn, Cu, Ti and Sr  
 156 were higher in the tree samples (**Fig 5C**). As regards samples from vines, high contents of Rb, Fe  
 157 and Zn were detected (**Fig 5D**).



158  
 159 **Fig. 5.** Variation of mineral contents in seed coats with respect to plant type: (A) total mineral content;  
 160 (B) major elements; (C) Al, Cl and Fe; (D) trace elements. V, T and H stand for vine, tree and herb,  
 161 respectively.

162  
 163 On the basis of their mineral contents, the seed coats from the 24 species under study were  
 164 categorized into two groups by using cluster analysis (**Fig. 6**). Group-I consisted of 19 species,  
 165 and the other 5 species were included in group-II, in such a way that the mean concentration  
 166 value of  $\Sigma_{M15}$  in the seed coats that belonged to group-II was at least twice that of group-I ones.



<b>Mg</b>	0.13	0.09	1.00																	
<b>Al</b>	0.21	0.00	0.97	1.00																
<b>P</b>	-0.70	-0.18	0.22	0.00	1.00															
<b>S</b>	0.23	0.58	0.72	0.54	0.50	1.00														
<b>Cl</b>	0.95	0.95	0.09	0.08	-0.47	0.41	1.00													
<b>K</b>	-0.06	-0.33	0.88	0.94	0.04	0.30	-0.23	1.00												
<b>Ca</b>	0.50	0.46	0.92	0.88	0.01	0.80	0.49	0.68	1.00											
<b>Rb</b>	0.91	0.54	0.39	0.52	-0.75	0.19	0.75	0.33	0.65	1.00										
<b>Sr</b>	0.41	-0.07	0.70	0.85	-0.50	0.11	0.17	0.84	0.68	0.75	1.00									
<b>Ti</b>	0.18	-0.26	0.74	0.89	-0.32	0.10	-0.06	0.93	0.63	0.57	0.97	1.00								
<b>Mn</b>	0.71	0.90	0.50	0.40	0.00	0.83	0.84	0.07	0.78	0.59	0.20	0.06	1.00							
<b>Fe</b>	0.13	-0.21	0.86	0.96	-0.14	0.28	-0.06	0.98	0.73	0.51	0.93	0.98	0.16	1.00						
<b>Cu</b>	0.11	-0.30	0.79	0.91	-0.22	0.15	-0.12	0.97	0.65	0.51	0.94	0.99	0.05	0.99	1.00					
<b>Zn</b>	0.23	0.00	-0.87	-0.73	-0.67	-0.81	0.14	-0.67	-0.71	0.07	-0.27	-0.39	-0.40	-0.58	-0.48	1.00				
<b>Pb</b>	0.11	-0.29	0.80	0.92	-0.21	0.16	-0.12	0.97	0.65	0.50	0.94	0.99	0.06	0.99	1.00	-0.49	1.00			

179 TPh = Total polyphenol content, Fla = Flavonoid content

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### 181 Comparison of minerals contents in seed coats and seed kernels

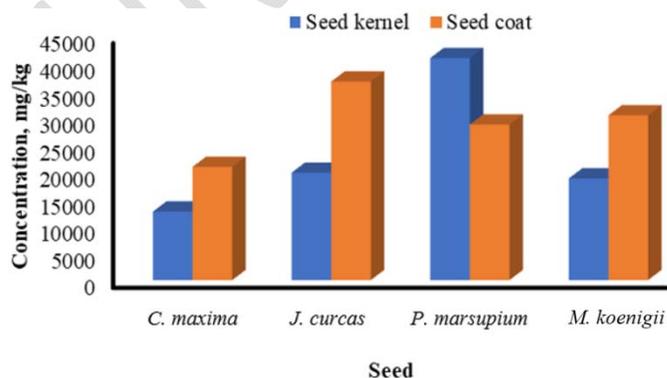
182 The minerals content in the seed coats and kernels from four of the species with the highest  
 183 mineral contents are shown in **Table 4**. Fifteen elements were detected in the coats, while in the  
 184 kernels only ten elements were identified. Generally, higher concentrations were detected in the  
 185 seed coats than in the seed kernels (**Fig. 7**).

186

187 **Table 4.** Distribution of elements in the seed kernel, mg/kg.

Species	Mg	P	S	K	Ca	Mn	Fe	Cu	Zn	Rb	Sr
<i>C. maxima</i>	1130	5505	1614	3920	102	63	157	14	78	11	2
<i>J. curcas</i>	1905	5513	1596	8032	2509	26	96	10	42	16	2
<i>P. marsupium</i>	5555	6743	5993	17119	5095	41	145	39	50	20	11
<i>M. koenigii</i>	551	1436	525	13130	2891	10	110	8	15	4	5

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189

190 **Fig. 7.** Distribution of total elements in seed coats and kernels.

191

## 192 **CONCLUSIONS**

193 Seed coats are major sources of polyphenols and minerals, with concentrations at least twice  
194 those found in the seed kernels. Remarkably high total polyphenol contents (of up to 32300  
195 mg/kg) were detected in the seed coats from tree species of the Loganiaceae, Phyllanthaceae,  
196 Lauraceae and Rutaceae families, while the highest flavonoid concentrations (of up to 26900  
197 mg/kg) corresponded to seed coats from the latter two families. As regards mineral contents, the  
198 highest total values were observed in the seed coats from three families: Lauraceae, Rutaceae and  
199 Euphorbiaceae. The highest concentrations of major elements (P, S, Mg, Ca and Al) were  
200 observed in seed coats from herb species, while those of Cl, K, Mn, Cu, Ti and Sr were higher in  
201 the tree samples. In turn, samples from vines featured high contents of Rb, Fe and Zn. Seed coats  
202 from Indian-laurel and curry tree stand out as particularly promising phytochemical and nutrient  
203 sources.

## 204 **CONSENT**

205 Not applicable.

## 206 **ETHICS APPROVAL**

207 Not applicable.

## 208 **CONFLICT OF INTEREST**

209 The authors declare no conflict of interest.

210

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