Original Research Article

Phenolic and mineral characteristics of seed coats and kernels

ABSTRACT

Aims: The objective of the present work is the investigation of the physico-chemical 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 weighted, after which seed coats were manually peeled and weighed separately. 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. Where as 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. Elements: 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.

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

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

INTRODUCTION

The seed coat protects the internal parts of the seed from fungi, bacteria and insects, and prevents water loss. It is composed of cellulose, fiber, polyphenols, starch, wax, etc. Its outer layer, called testa, is generally hard and thick, while its inner layer, known as the tegmen, is softer [1]. Enrichment of various compounds (viz. minerals, cellulose, fiber, polyphenols, starch, wax, etc.) in seed coats has been reported in the literature [2,3,4,5,6,7]. Among these phytochemicals: polyphenols have become the subject of increasing research efforts owing to their potential beneficial effects on human health [8].

Among the plants found in Raipur area, Black Siris (*Albizia odoratissima* (L.f.) Benth.), Malabar spinach (*Basella rubra* L., syn. *Basella alba* L.), wax gourd (*Benincasa hispida* (Thunb.) Cogn.), squash (*Cucurbita maxima* (Duchesne) Duchesne ex Poir.), watermelon (*Citrullus lanatus* (Thunb.) var. *lanatus*), Persian melon (*Cucumis melo* var. *cantalupo* Ser.), Liane Cacorne (*Entada gigas* (L.) Fawc. & Rendle), tree cotton (*Gossypium arboreum* L.), physic nut (*Jatropha curcas* L.), Persian walnut (*Juglans regia* L.), hyacinth bean (*Lablab purpureus* (L.) Sweet), calabash (*Lagenaria siceraria* Standl.), Chinese-okra (*Luffa acutangula* Roxb.), sponge gourd (*Luffa aegyptiaca* Mill.), Indian-laurel (*Litsea glutinosa* (Lour.) C.B.Rob.), Indian-lilac (*Melia azadirachta* L., syn. *Azadirachta indica* A.Juss.), bitter melon (*Momordica charantia* L.), curry tree (*Murraya koenigii* Spreng.), emblic (*Phyllanthus emblica* L.), East Indian kino (*Pterocarpus marsupium* Roxb.), Indian sandalwood (*Santalum album* L.), Ceylon-oak (*Schleichera oleosa* (Lour.) Oken), clearing-nut-tree (*Strychnos potatorum* L.f.), and Indian tuliptree (*Thespesia* *populnea* Sol. ex Corrêa) are widely used as medicine, food and fodder for animals [9,10,11,12,13,14,15,16,17,18,19,20,21,22].

Accumulation of the nutrients and polyphenols in some seed coats were reported [6, 23, 24, 25, 26, 27]. In this work, the physical and chemical characteristics of the seed coats and kernels from these 24 species (2 herbs, 11 vines and 11 trees) are analyzed, with emphasis on their polyphenol contents.

METHODS AND MATERIALS

Sample collection and handling

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

Analyses

Sigma-Aldrich AR grade reagents were used for analysis of polyphenols. 0.1 g of powdered seed coat were extracted with an acetone:water mixture (7:3, v/v), as recommended by Bertaud et al. [28]. An appropriate fraction was allowed to react with Folin-Ciocalteu reagent for color development, and absorbance was measured at λ=740 nm with a UV-1800 (Shimadzu, Kyoto, Japan) UV-Vis spectrophotometer [29]. Three replicates for each solvent extract were performed to determine the total phenolic content (TPh), which was expressed in terms of tannic acid equivalents by using a standard calibration curve. For flavonoid (Fla) analysis, a fraction of the extract was reacted with an aluminum chloride solution to develop a yellow colored complex, measuring the absorbance at λ=410 nm [30]. The Fla concentration was determined with the aid of standard quercetin calibration curve and indicated in terms of quercetin equivalents. Three replicates for each solvent extract were performed, and results are presented as average values across the three replicates.

A III Tracer-SD portable XRF (Bruker, Billerica, MA, USA) spectrophotometer was used for the quantification of 15 elements: K, Rb, Mg, Ca, Sr, Al, P, S, Cl, Ti, Mn, Fe, Cu, Zn and Pb. Standard brown and white cowpea (*Vigna unguiculata* (L.) Walp.) seeds were used as reference material to standardize the analyte concentration [31].

Statistical analyses

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

RESULTS AND DISCUSSION

Physical characteristics

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

Fig. 1. Seed samples from: (1) *Phyllanthus embilica*, (2) *Pterocarpus marsupium*, (3) *Luffa aegyptiaca*, (4) *Benincasa hispida*, (5) *Citrullus lanatus*, (6) *Cucumis melo*, (7) *Cucurbita maxima*, (8) *Luffa*

acutangula, (9) *Lagenaria siceraria*, (10) *Momordica charantia*, (11) *Litsea glutinosa*, (12) *Gossypium arboreum*, (13) *Thespesia populnea*, (14) *Santalum album*, (15) *Murraya koenigii*, (16) *Schleichera oleosa*, (17) *Melia azadirachta*, (18) *Strychnos potatorum*, (19) *Albizzia odoratissima*, (20) *Basella rubra*, (21) *Lablab purpureus*, (22) *Jatropha curcas*, (23) *Juglans regia*, and (24) *Entada gigas*.

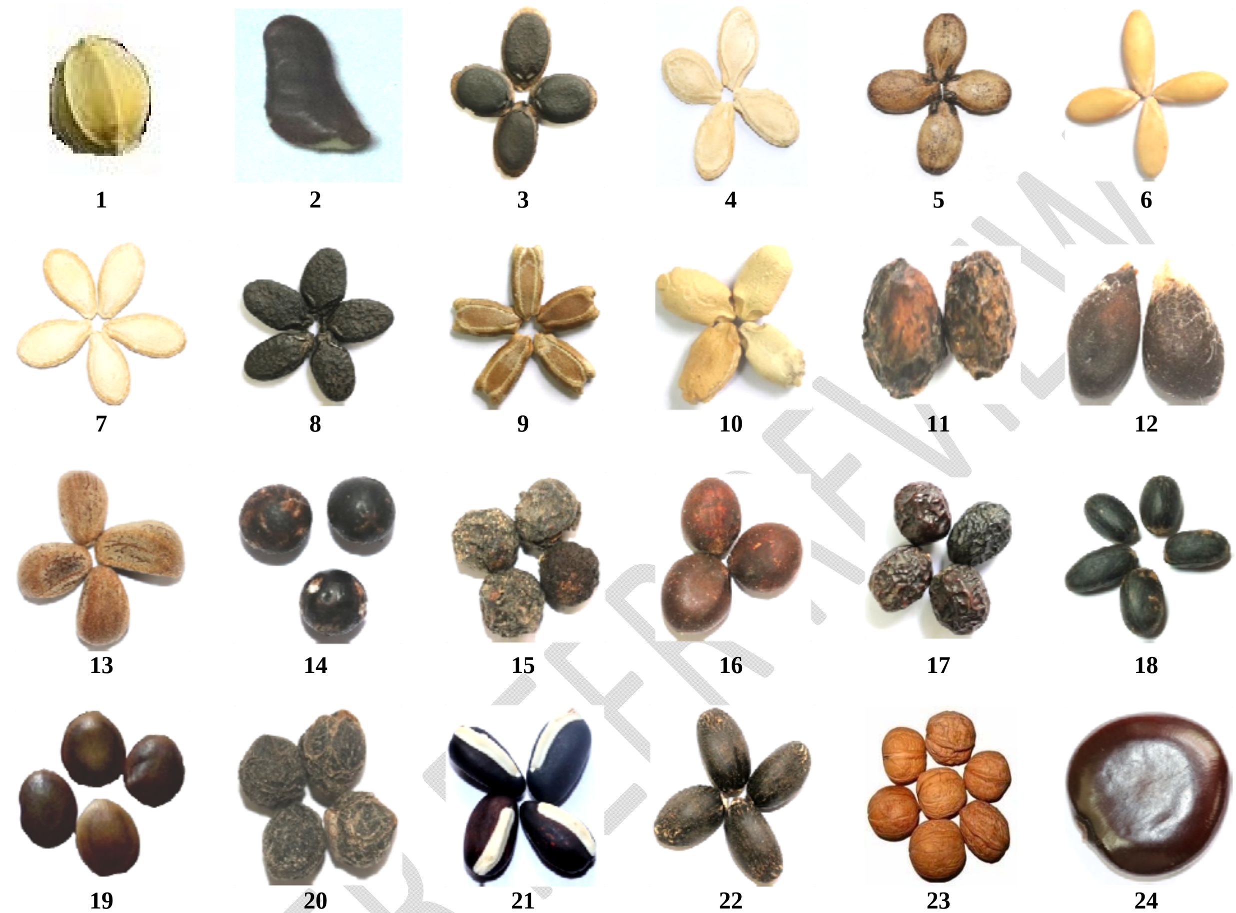


Table 1. Physico-chemical characteristics of seeds and seed coats. Total phenolic contents and flavonoid contents correspond to the seed coat and kernel samples.

Species	Family	Type	Seed mass (mg)	Color	Seed coat (%)	Seed coat, mg/kg		Kernel, mg/kg	
						TPh	Fla	TPh	Fla
Basella rubra	Basellaceae	V	38	BrB	47±2	11400	10500	3457	1650
Cucurbita maxima	Cucurbitaceae	V	132	YeW	18±1	3100	1900	4931	1100
Lagenaria siceraria	Cucurbitaceae	V	216	WhBr	42±2	14400	8900	1956	1400
Citrullus lanatus	Cucurbitaceae	V	38	ReBr	49±2	18500	13100	2278	1280
Luffa aegyptiaca	Cucurbitaceae	V	105	B	43±2	3100	2500	780	620

Cucumis melo	Cucurbitaceae	V	25	LY	28±1	2900	2100	965	300
Luffa acutangula	Cucurbitaceae	V	122	B	47±2	8300	7200	2144	1380
Benincasa hispida	Cucurbitaceae	V	64	YeW	47±	2900	2600	4074	2280
Momordica charantia	Cucurbitaceae	V	189	YeBr	35±1	30847	1700	1769	1180
Jatropha curcas	Euphorbiaceae	H	758	Br	47±2	14700	4000	1501	4260
Lablab purpureus	Fabaceae	V	293	DBr	34±1	22000	3700	1260	2550
Albizia odoratissima	Fabaceae	T	159	LBr	42±	27000	4100	2492	4300
Entada gigas	Fabaceae	V	23623	DBr	40±1	26900	3900	18840	2650
Pterocarpus marsupium	Fabaceae	T	933	LY	93±3	25800	3800	31760	5800
Juglans regia	Juglandaceae	T	12200	PY	32±1	9600	1900	1045	1520
Litsea glutinosa	Lauraceae	T	248	DBr	43±2	29200	26900	4931	3880
Strychnos potatorum	Loganiaceae	T	280	B	24±1	26000	15000	2707	1640
Gossypium arboreum	Malvaceae	H	82	Br	48±2	4000	3500	7263	7160
Thespesia populnea	Malvaceae	T	162	LBr	47±2	16800	8000	15839	12020
Melia azadirachta	Meliaceae	T	972	DBr	65±2	1800	1200	1822	1300
Phyllanthus embilica	Phyllanthaceae	T	920	PW	95±3	27000	3500	4476	3750
Murraya koenigii	Rutaceae	T	155	B	12±1	32300	25300	3457	3650
Santalum album	Santalaceae	T	180	DBr	40±2	10900	6200	7075	2750
Schleichera oleosa	Sapindaceae	T	352	DBr	49±2	8500	5400	2198	1950

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

Polyphenol contents

The concentration of TPh and Fla in the seed coats and kernels varied from 1800 to 32300 mg/kg, from 780 to 31760 mg/kg, from 1200 to 26900 mg/kg and from 300 to 12020 mg/kg, respectively with a mean value of 15748, 5376, 6954 and 2932 mg/kg (**Table 1**). The [TPh]/[Fla] ratio in the studied seed coats and kernels ranged from 1.1 to 18.1 and 0.4 to 7.1 with a mean value of 3.5 and 1.9, respectively. Higher contents of TPh and Fla in the seed coats than the seed kernels was marked.

Large variations in the polyphenol content were observed from one species to another, with noticeably higher TPh and Fla values in seed coats and kernels from tree species (**Fig. 2**). Similarly, remarkable differences in the polyphenol content of the seed coat and kernel samples were detected as a function of the family (**Fig. 3**). In the case of seed coats, four families showed the highest TPh contents: Loganiaceae, Phyllanthaceae, Lauraceae and Rutaceae. Whereas in the case of kernels, three families exhibited the highest TPh contents: Fabaceae, Malvaceae and Santalaceae. As regards Fla contents, the highest concentrations corresponded to seed coats from Lauraceae and Rutaceae families.

Mineral contents

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

P and K nutrients were abundant in the seed coats, ranging from 99 to 4983 mg/kg and from 1714 to 21982 mg/kg, respectively. The highest P contents was observed in seed coats from Cucurbitaceae family, while the highest K contents (>15000 mg/kg) were detected in seed coats from *Pterocarpus marsupium*, *Litsea glutinosa*, *Thespesia populnea* and *Murraya koenigii*.

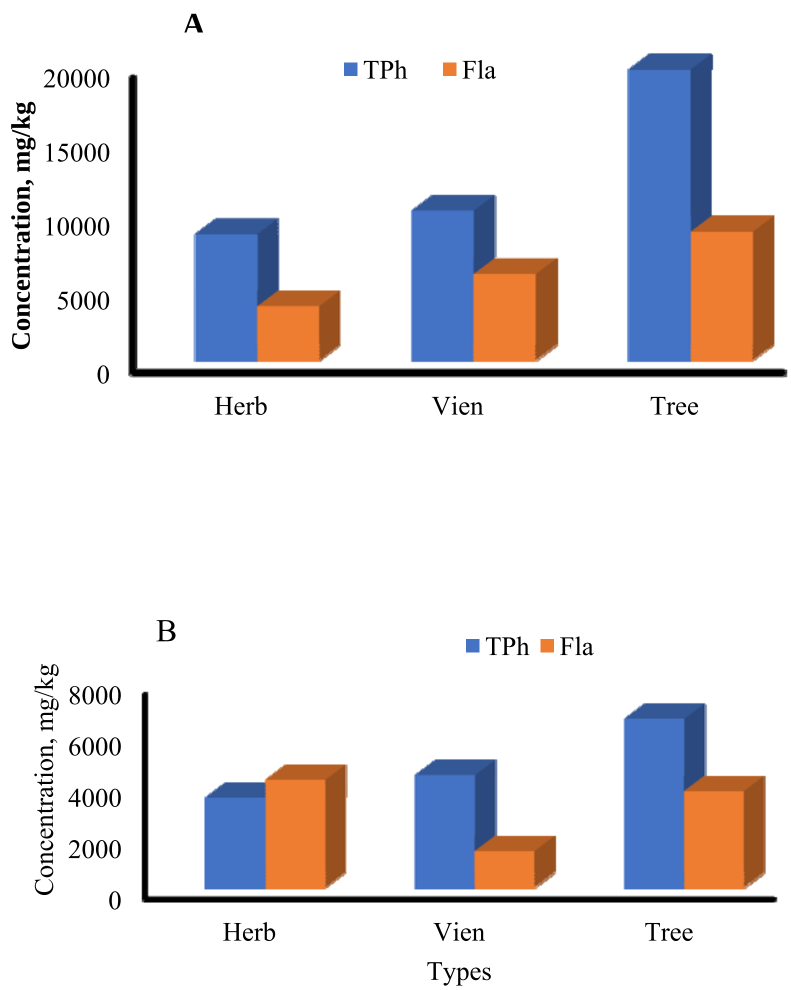
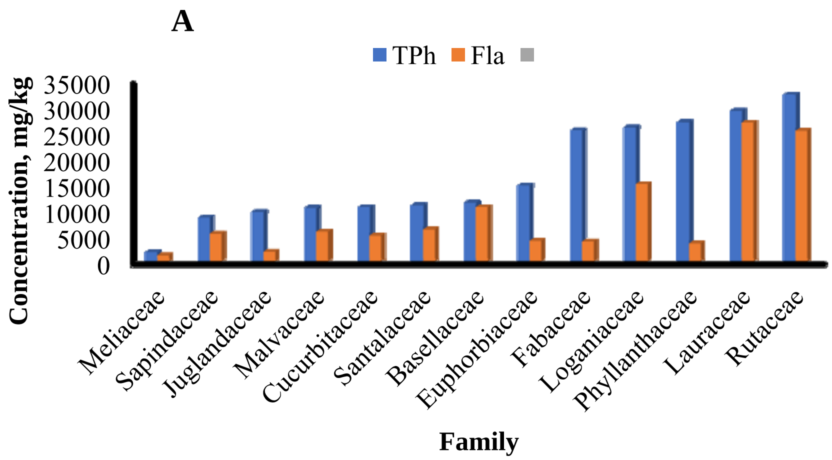


Fig. 2. Polyphenol concentration variation in seed coats with respect to plant types. (A): Seed coats and (B): Seed kernels.



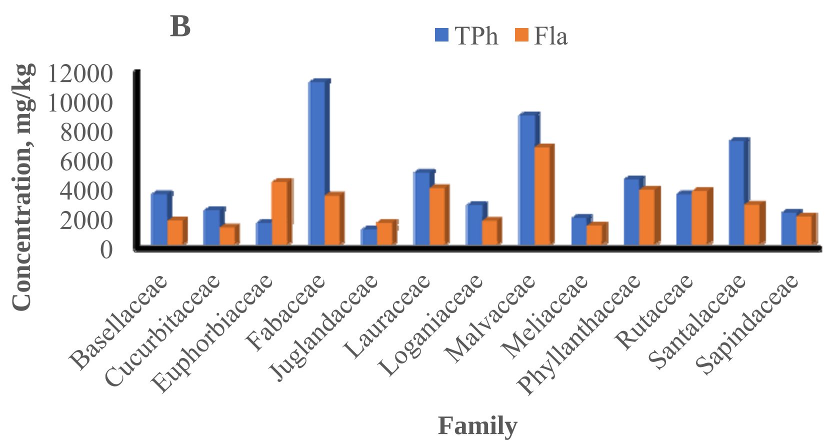


Fig. 3. Polyphenol concentration variation of seed coats with respect to family. (A) seed coat, (B) seed kernel

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
B. rubra	1012	78	99	194	71	6305	3409	12	4	39	71	389	4	33	3

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

S and Cl concentrations in seed coats were in the 116–3140 mg/kg and in the 48–4859 mg/kg range, respectively. The highest values for S and Cl corresponded to *Albizzia odoratissima* and *Thespesia populnea*, respectively.

Mg and Ca elements, probably present as silicates, ranged from 105 to 5808 mg/kg and from 338 to 14210 mg/kg, respectively. In this case, the highest concentrations of Mg and Ca were observed in seed coats from *Strychnos potatorum* and *Jatropha curcas*.

The concentrations of other elements in the seed coats, expressed in mg/kg, were in the following ranges: 1–26 (Rb), 1–32 (Sr), 35–844 (Al), 7–39 (Ti), 14–132 (Mn), 65-685 (Fe), 1–914 (Cu), 1-49 (Zn) and 1–3 (Pb). The highest concentrations of Rb, Sr, Al, Ti, Pb, Mn, Fe, Cu and Zn were found in the seed coats from *Santalum album*, *Citrullus lantus*, *Melia azadirachta*, *Basella rubra*, *Pterocarpus marsupium*, *Albizzia odoratissima*, *Melia azadirachta*, *Pterocarpus marsupium* and *Cucumis melo*, respectively. It is worth noting that seed coats from *Thespesia populnea* featured high contents of Cl, K, Fe and Cu.

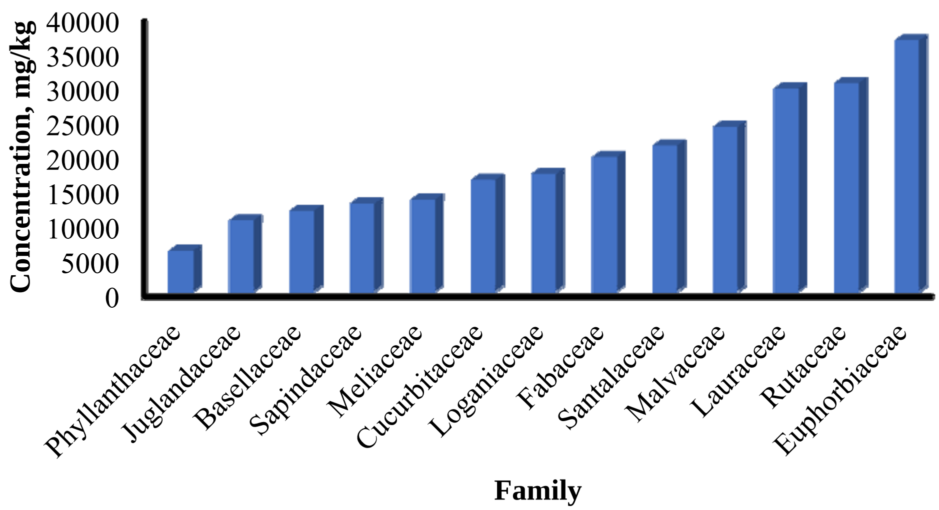


Fig. 4. Total mineral content familywise.

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

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

Correlation coefficients

The correlation coefficients (r) for the seed coat samples from species belonging to the Fabaceae family are shown in **Table 3**. Good correlations were found among K, Mg, Ca, Al, Sr, Ti, Fe, Cu and Pb, suggesting similarities in their bioaccumulation. Between TPh and Fla contents and Cl, Rb and Mn were well correlated, which would point to their accumulation via bond formations with phenolic groups. In addition, good correlations were observed among S, Mg, Ca and Mn, which may be ascribed to the accumulation of the latter three as sulfur compounds.

Distribution of minerals in seed coats and seed kernels

The content of the minerals in the seed coat and the kernels of the prominent seeds is shown in **Tables 4**. The nutrients are well abundant in the seed coats than the kernels. Fifteen elements were detected in the coats. However, in the kernels, only ten elements were identified. Generally, higher concentration of nutrients in the seed coats than the kernel was marked, **Fig. 6**.

(A) **(B)**

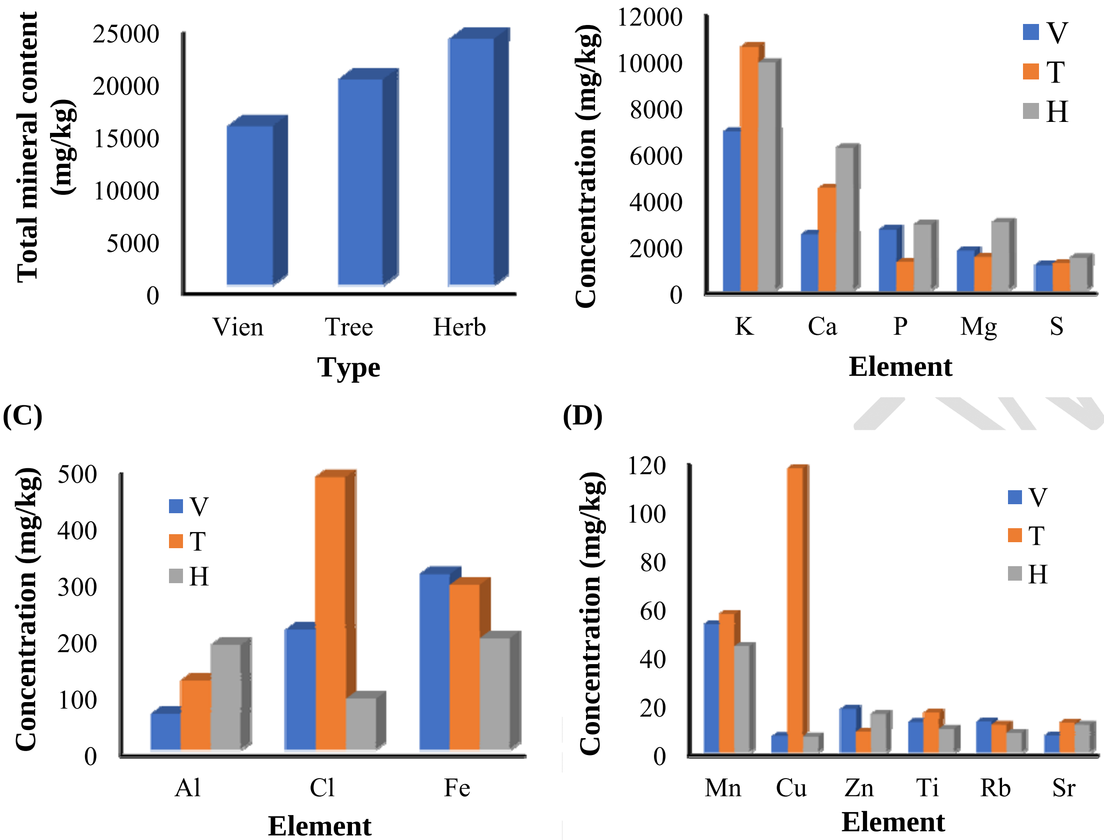


Fig. 5. Variation of mineral contents in seed coats with respect to plant type: (A) total mineral content; (B) major elements; (C) Al, Cl and Fe; (D) trace elements.

Table 3. Correlation coefficients (r) among various constituents of the Fabaceae family seed coat samples.

	TPh	Fla	Mg	Al	P	S	Cl	K	Ca	Rb	Sr	Ti	Mn	Fe	Cu	Zn	Pb
TPh	1.00																
Fla	0.80	1.00															
Mg	0.13	0.09	1.00														
Al	0.21	0.00	0.97	1.00													
P	-0.70	-0.18	0.22	0.00	1.00												
S	0.23	0.58	0.72	0.54	0.50	1.00											
Cl	0.95	0.95	0.09	0.08	-0.47	0.41	1.00										
K	-0.06	-0.33	0.88	0.94	0.04	0.30	-0.23	1.00									
Ca	0.50	0.46	0.92	0.88	0.01	0.80	0.49	0.68	1.00								

Rb	0.91	0.54	0.39	0.52	-0.75	0.19	0.75	0.33	0.65	1.00							
Sr	0.41	-0.07	0.70	0.85	-0.50	0.11	0.17	0.84	0.68	0.75	1.00						
Ti	0.18	-0.26	0.74	0.89	-0.32	0.10	-0.06	0.93	0.63	0.57	0.97	1.00					
Mn	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				
Fe	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			
Cu	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		
Zn	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	
Pb	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

TPh = Total polyphenol content, Fla = Flavonoid content

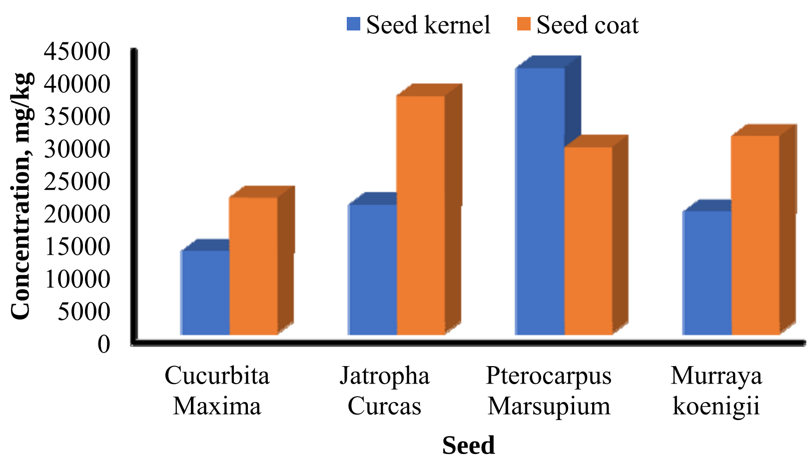


Fig. 6. Distribution of total elements in seed coats and kernels.

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

Seed	Mg	P	S	K	Ca	Mn	Fe	Cu	Zn	Rb	Sr
Cucurbita maxima	1130	5505	1614	3920	102	63	157	14	78	11	2
Jatropha curcas	1905	5513	1596	8032	2509	26	96	10	42	16	2
Pterocarpus marsupium	5555	6743	5993	17119	5095	41	145	39	50	20	11

Murraya koenigii	551	1436	525	13130	2891	10	110	8	15	4	5

CONCLUSIONS

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

CONSENT

Not applicable.

ETHICS APPROVAL

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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