Original Research Article

Evaluation of the Phytochemical and Mineral Characteristics of some Selected Sapotaceae Plants

Suryakant Chakradhari[1], Manas Kanti Deb[2], Khageshwar Singh Patel[2*], Pablo Martín-Ramos[3], Erick K. Towett[4] and Jesús Martín-Gil[5]

[1]School of Studies in Environmental Science, Pt. Ravishankar Shukla University, Raipur-492010, India: suryachakradhari99@gmail.com.

[2]School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur-492010, India: debmanas@yahoo.com.

[2*]School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur-492010, India; patelkhagrshwarsingh@gmail.com.

[3]Department of Agricultural and Environmental Sciences, EPS, Instituto de Investigación en Ciencias Ambientales de Aragón (IUCA), University of Zaragoza, Carretera de Cuarte, s/n, 22071 Huesca, Spain; pmr@unizar.es.

[4]World Agroforestry Centre, PO Box 30677, Nairobi, 00100, Kenya; e.towett@cgiar.org

[5]Agriculture and Forestry Engineering Department, ETSIIAA, Universidad de Valladolid, Avenida de Madrid 44, 34004 Palencia, Spain; mgil@iaf.uva.es.

*Corresponding author

AUTHORS' CONTRIBUTIONS

S.C. collected the plant samples, prepared and preserved them, and analyzed the starch, polyphenol, flavonoid and oil contents. M.K.D. collected the surface soil samples and measured their physical parameters. K.S.P. designed the investigation and coordinated the analyses and paper writing. E.K.T. conducted the XRF measurements. J.M.-G. and P.M.-R. carried out the FTIR characterization and thermal analyses of the samples.

K.S.P. and P.M.-R. wrote the original draft. P.M.-R. took care of the manuscript review and editing.

ABSTRACT

Aims: To study the spectral and thermal characteristics, and the oil, starch, polyphenol and mineral contents of seeds and leaves from three Sapotaceae species, provided that trees and shrubs of this family are an important source of nutritional and functional products.

Methodology: Leaves and seeds from three Sapotaceae plants, namely Moa tree (*Madhuca indica* J. F. Gmel.), Chico sapote (*Manilkara zapota* (Linn.) van Royen) and Spanish cherry (*Mimusops elengi* Linn.), were collected in the Raipur area of Chhattisgarh, India. Their physicochemical characterization (including oil, polyphenol, starch and mineral contents; functional groups; and thermal degradation patterns) was carried out by using various techniques, viz. solvent extraction, spectrophotometry, enzymatic digestion, X-ray fluorescence (XRF) and Fourier-transform infrared (FTIR) spectroscopies, thermogravimetric/derivative thermogravimetric (TG/DTG) and differential scanning calorimetry (DSC), respectively.

Results: The three Sapotaceae seeds under study were found to contain polyphenol, mineral, starch and oil contents in the 1850–23180 mg/kg, 11390–19385 mg/kg, 6.7–9.1% and 9.8-54.1% range, respectively. Their leaves and seed coats featured total phenolic contents in the 24260–28600 mg/kg and 7810–23060 mg/kg range, respectively, and mineral contents in the 8823–27462 mg/kg and 3619–15884 mg/kg

range, respectively. The functional groups of the phytochemicals, studied by FTIR, were assigned. Their thermal decomposition patterns, which involve loss of water and volatile organic compounds, proteins, oil and starch/cellulose, were also described.

Conclusion: The Sapotaceae leaves, seed coat, kernel and cake are enriched with very high contents of starch, proteins, polyphenols and minerals, suggesting their possible valorization in human food, animal feeding and as herbal medicines.

Keywords: FTIR, oil, polyphenol, Sapotaceae, starch, thermal analysis, XRF.

1. INTRODUCTION

The Sapotaceae are a family of flowering plants belonging to order Ericales. They are deciduous trees widespread across India with a wide range of local uses as a food source and in Ayurvedic medicine (**1, 2, 3, 4, 5, 6, 7**). Their pharmacological properties should be referred to their content in sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides.

In this work, three representative trees from this family, namely *Madhuca indica* J. F. Gmel. (*Madhuca longifolia* (Koenig) J. F. Macb. var. latifolia (Roxb.) Cheval., syn. *Madhuca latifolia* Macb., *Bassia latifolia* Roxb.), *Manilkara zapota* (Linn.) van Royen, and *Mimusops elengi* (Linn.) are studied.

Moa, Mahua or Butternut tree *(M. indica*) shows multiple applications: its flowers were used as a seasonal grain substitute (**8**), cooling agent, tonic, aphrodisiac, and for the treatment of helminths; its bark is used as decoction for rheumatism, bleeding and spongy gums; its leaves were used in verminosis and gastropathies treatments (**9**); and

its seeds were of economic importance as a good source of edible fats (**10**). Chico sapote (*M. zapota*) produces edible fruits and was also the source of *chicle*, a chewing gum component. Moreover, its leaves exhibited antihyperglycemic, hypocholesterolemic and antioxidant activities (**11**). As regards Bakul (*M. elengi*), *gutta percha* (a trans-1,4-polyisoprene) and dental products were obtained from its latex, and its extracts had been reported to possess antibacterial, antifungal, anti-cariogenic, free radical scavenging, antihyperglycemic, antineoplastic, gastroprotective, antinociceptive and diuretic effects (**12**). Furthermore, the oils from the seeds of the three trees under study have been reported to have potential uses in biodiesel production (**13, 14**).

Total phenolic and polyphenol contents of *M. zapota* and *M. elengi* leaves and kernels had been reported in the literature (**15, 16, 17**), and, more recently, seventy-two volatile compounds were identified in the headspace of *M. zapota* fruits (**18**).

In order to complete the data reported to date in the literature, the aim of this article is to characterize the seeds and leaves of *M. indica*, *M. zapota* and *M. elengi* by rapid techniques of structural and thermal elucidation (ATR-FTIR, TG and DSC) and to evaluate their contents in trace elements, starch and polyphenols. The former goal is a requisite for the commercialization of Sapotaceae-derived products, and the latter is of key importance with a view to their pharmacological applications.

2. MATERIALS AND METHODS

2.1. Plant Collection

The plant samples from *M. indica, M. zapota* and *M. elengi* were botanically authenticated with the aid of standard monographs (**19**). Their leaves and fruits were collected from the Raipur area of Chhattisgarh, India (21°15′0″ N, 81°37′48″ E) in May-July 2016. They were stored in plastic bags and transported to the laboratory. The seeds from the fruits were manually separated.

The plant leaves were washed with the de-ionized water and air-dried. They were sundried in a glass room for one week, then further dried for 24 h at 50 ^{0}C in an oven, and finally stored in glass containers at -4 ^{0}C in a deep freezer till the analyses were conducted.

2.2. Physical Parameters

The mass of randomly selected biomass samples was determined by weighing with an AY120 electronic precision balance (Mettler Toledo, Columbus, OH, USA; ±0.0001 g). Bulk density (in kg/m^3) of the biomass was determined by the toluene displacement method (**20**).

The biomass sample was ground with a universal laboratory mill to pass through a sieve of mesh size ≤ 0.10 mm, finally getting a fine powder.

The moisture content of the biomass was determined by drying samples in triplicate at 105 ^{0}C in an air oven for 6 hr prior to the analysis, and mean values are reported. All characterization results are presented on a dry weight (dw) basis.

2.3. Oil Content Analysis

To determine oil contents, 5.0 g of dried seeds were agitated with 25 mL of *n*-hexane in a centrifuge tube in a Vortex REAX Top shaker (Heidolph, Schwabach, Germany) at 2500 rpm for 1 min, as described by Górnaś (**21**). The combined supernatants were evaporated in a rotary vacuum evaporator at 40 ^{0}C until constant weight was reached. The oil content was expressed in % (w/w) on the seed dry weight (dw) basis.

2.4. Starch Content Analysis

AR grade regents, including sodium maleate (CAS 371-47-1) buffer, sodium acetate (CAS 371-47-1) buffer, potassium hydroxide (CAS 1310-58-3), amyloglucosidase (CAS 9032-08-0), pancreatic-α-amylase (MDL MFCD00081319) and glucoseoxidase–peroxidase, were purchased from Megazyme International Ireland Ltd. and were used for the starch analysis.

The starch content of seed kernel was analyzed by the enzymatic digestion method (**22**). Amylase and amyloglucosidase were used for the hydrolysis of the soluble starch, which was carried out at 37 ^{0}C for 16 hr. The hydrolysis of the resistant starch was performed in an acetate buffered KOH solution. The glucoseoxidase–peroxidase reagent was employed for the spectrophotometrical measurement of the resulting glucose.

2.5. Phenol Content Analysis

AR-grade Folin-Ciocalteu reagent, aluminum chloride (CAS 7446-70-0), tannic acid (CAS 1401-55-4) and quercetin (CAS 6151-25-3) were employed for the phenolic content analysis, all purchased from Sigma–Aldrich. 100 mg of powdered sample were extracted with 5 mL of an acetone:water (70:30, v/v) solution in an ultra-sonic bath for

20 min at 20 ^{0}C. Then, 5 mL of a fresh acetone:water (70:30, v/v) solution were added to the mixture and the extraction was repeated for 20 min at 20 ^{0}C. After centrifugation, the supernatant was collected. The total phenolic content of each extract was determined as tannic acid equivalents by using the Folin-Ciocalteu reagent, according to the method of Singleton and Orthofer (**23**). The flavonoid content was determined by the aluminum chloride method as quercetin equivalents (**24**). The analyses were conducted in triplicate.

2.6. Mineral Content Analysis

The X-ray fluorescence (XRF) analysis of the elements present in the samples was carried out in triplicate by using a Bruker III Tracer SD T3S2731 (Kennewick, WA, USA) spectrometer equipped with a 4W rhodium anode and Xflash SDD with 2028 channels. The calibration was carried out by using standard brown and white cowpea seeds and mango leaves and pulp samples.

2.7. Vibrational and Thermal Characterization

The vibrational spectra in the 400-4000 cm^{-1} spectral range 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, with a 1 cm^{-1} spectral resolution and averaging 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 the samples

150 in a slow stream of N_2 (20 mL·min^{-1}) from room temperature up to 800 ^{0}C, with a
151 heating rate of 20 ^{0}C·min^{-1}. Pyris v.11 software was used for data analysis.

152 **2.8. Statistical Analyses**

153 An ANOVA was conducted to compare TPh, Fla, oil and starch contents in the various
154 plant parts. Tukey's multiple range test at 0.05 probability level (P<0.05) was chosen
155 for the *post hoc* comparison of means. Pearson's correlation test was used in order to
156 assess the association among the quantitative variables under study (viz. oil, soluble
157 starch, resistant starch, TPh, Fla, K, Rb, Mg, Ca, Sr, Al, P, S, Cl, As, Ti, V, Mn, Fe, Co,
158 Cu, Zn, Mo and Pb contents). The statistical analyses were conducted in IBM SPSS
159 software .

160 **3. RESULTS AND DISCUSSION**

161 **3.1. Physical Characteristics**

162 The physical characteristics of *M. indica* (MI), *M. zapota* (MZ) and *M. elengi* (ME)
163 samples are summarized in **Table 1**. Their leaves were dark-green colored, with
164 different shapes, as shown in **Fig. 1**. Seeds were light-brown colored, with a light
165 yellow-colored kernel. The microscopic images of the MI, MZ and ME leaves, kernels
166 and seed coats are presented in **Fig. 2**. The kernel samples seemed to be amorphous,
167 unlike the seed coat and leaves samples. The average leaf mass for MI, MZ and ME was
168 2453±48 mg, 926±22 mg and 1365±25 mg (dw), respectively. The average mass per
169 single seed of MI, MZ and ME was found to be 2235±43, 650±12 and 614±15 mg (dw),
170 respectively, out of which the kernel accounted for 71.1% (645±11 mg), 37.9% (403±7

mg) and 30.0% (430±8 mg) of the total seed weight, respectively. Similarly, kernel mass per single seed was 1590±32 mg, 247±5 mg and 184±4 mg for MI, MZ and ME seeds, respectively. Among the three species under study, a higher mass of both leaves and seeds was observed for MI. The bulk density of the leaf, seed kernel and seed coat samples varied from 512 to 684 kg/m^3, with a mean value of 595 kg/m^3. The moisture content varied from 2.2 to 6.2% and had good correlation (r = 0.94) with the mass of the respective biomass samples (**Table 1**).

Table 1. Physical characteristics of the samples from the selected Sapotaceae species.

Parameter	*Madhuca indica*			*Manilkara zapota*			*Mimusops elengi*		
	Leaves	Seed kernel	Seed coat	Leaves	Seed kernel	Seed coat	Leaves	Seed kernel	Seed coat
Color	Dark green	Light yellow	Light brown	Dark green	Light yellow	Dark brown	Dark green	Light yellow	Dark brown
Shape	Lanceolate-ovate	Elliptic	Oblong shaped	Elliptic-oval	Narrow-ovate	Oval shaped	Elliptic-oblong	Obovate	Oblong ellipsoid
Mass, mg	2453±48	1590±32	645±11	926±22	247±5	403±7	1365±25	184±4	430±8
BD, kg/m^3	581±12	684±13	615±11	602±10	614±13	567±10	613±12	512±10	564±13
Moisture content, %	6.2±0.2	4.3±0.2	3.4±0.2	3.8±0.2	2.7±0.1	3.1±0.2	4.4±0.2	2.2±0.1	3.9±0.1

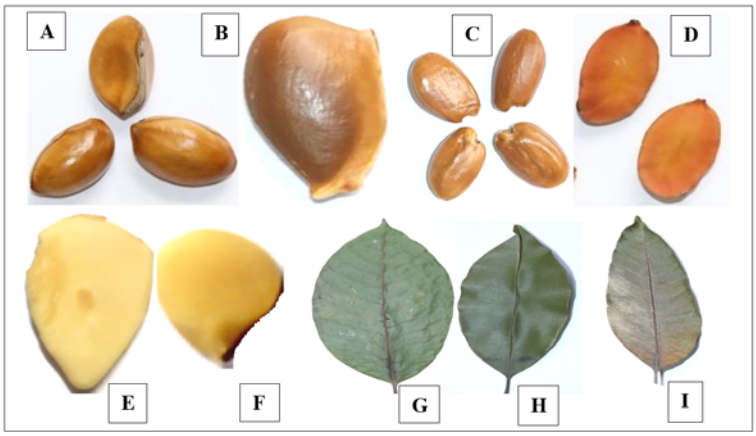


Fig. 1. Images of seed, kernel and leaves of *Madhuca indica* (A, D & G), *Manilkara zapota* (B, E & H) and *Mimusops elengi* (C, F & I).

185

186

187 **Fig. 2**. Optical microscope images at different magnifications for the different samples

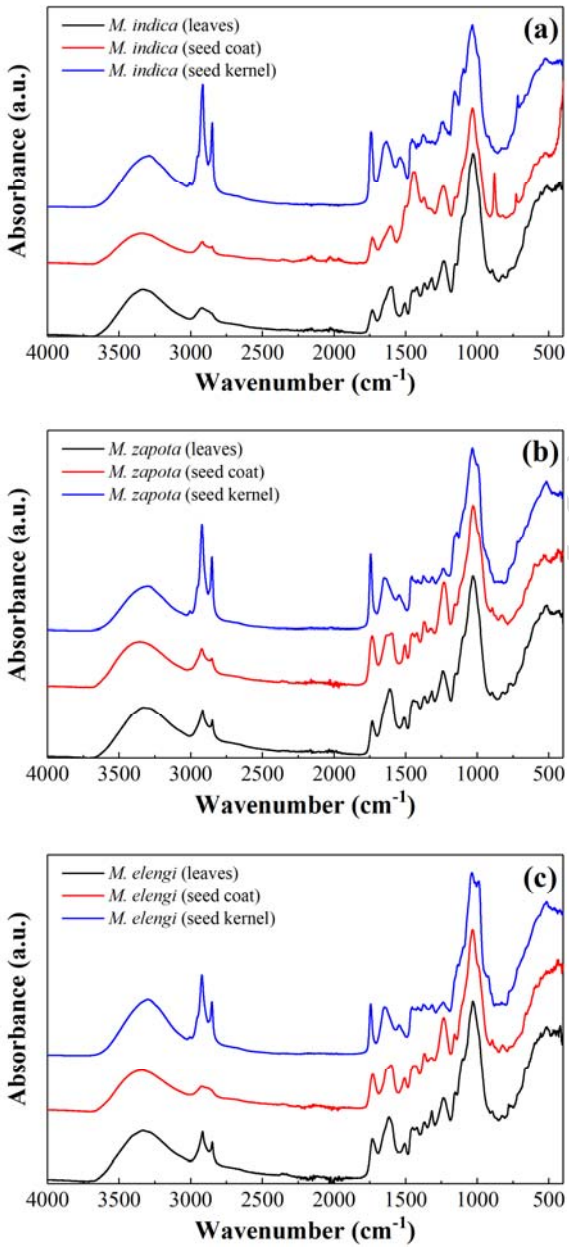
188 under study.

3.2. Vibrational Characteristics

The ATR-FTIR spectra for leaves, seed coat and seed kernel samples from the three species of the Sapotaceae family under study are depicted in **Fig. 3**. The corresponding assignments of the bands are summarized in **Table 2**. Peaks at around 3300 cm^{-1} (ν OH) corresponded to typical characteristic absorption from cellulose (**25**). Peaks at 2922-2917 cm^{-1} (–CH_2 aldehydic symmetrical stretching) and at 2854-2850 cm^{-1} (-CH stretching) indicated the presence of cutine and wax. Peaks at 1735 and at around 1370 cm^{-1} were indicative of hemicellulose, specifically of C=O stretching (1733 cm^{-1}) and -CH_3 symmetric deformation (1378-1369 cm^{-1}). Prominent bands in the 1340 to 890 cm^{-1} region were also attributed to cellulose: at 1336 cm^{-1} (δ CH in-plane), 1320-1316 cm^{-1} (C-H vibration), 1153-1147 cm^{-1} (ν C-O-C in bridge, asymmetric), 1038-1031 cm^{-1} (ν C-O or –C-O-C- stretching) and 896-894 cm^{-1} (ν C-O-C in bridge, symmetric, characteristic of the glycosidic ring in cellulose). The presence of pectin was indicated by peaks associated with COO- asymmetric and O-CH_3 stretching (at 1454-1445 cm^{-1}) for calcium pectate and with –CH_3 distortion (1242-1231 cm^{-1}) for pectic ester. The band that appeared at 1424-1416 cm^{-1} can be attributed either to cellulose (ρ CH_2, sym.) or to symmetric stretching vibration for calcium pectate (**26**). Bands at 831-819 cm^{-1} were due to aromatic C-H out-of-plane binding or to C-O-C deformation and they suggested the presence of D-Glc pyranoside configurations. Bands in the 776-717 cm^{-1}, assigned to O-C=O in-plane deformation or to a CH_2 rocking deformation, were attributed to phenolic components. For samples from leaves and seed coat, two bands attributed to lignin could be observed: the band of the aromatic ring stretching of the

lignin (1606 cm^{-1}), which appeared at 1617-1597 cm^{-1}; and the band of the aromatic skeletal vibration (C=C aromatic symmetrical stretching), at 1515-1507 cm^{-1}. This latter band did not appear in the spectra for seed kernel sample and its intensity divided by that of the band at 895-881 cm^{-1} (also missing for seed kernel) informed on the functionality of the lignin.

Seed kernel samples showed strong characteristic bands at around 1744 cm^{-1}, 1650 cm^{-1}, 1540 cm^{-1} and 950 cm^{-1}. The band at 1744 cm^{-1}, assigned to C=O (non-conjugated moieties vibrations) could be associated to the stretching vibration of the ester carbonyl functional groups of the triglycerides. The peak obtained at 1661-1634 cm^{-1} could be characteristic of C=C absorption cellulose when it is cross-linked and dehydrated, but may also be assigned to amide N–H & C=O stretching from mucilage (**27**) and to an enrichment in unsaturated oils. The presence of this band, typical of the vinyl group, justified the quantitative presence of unsaturated oils in the kernel of all the seeds under study. The sharp, intense C-H wags at 1000, 926 and 923 cm^{-1} were also indicative of vinyl. It is known that the *Sapotaceae* oil (**28**) (at least in the case of *Argania spinosa*) is obtained from the kernel and not from the seed coat.



227

228 **Fig. 3**. Comparison of the leaves (*black*), seed coat (*red*) and seed kernel (*blue*) ATR-

229 FTIR spectra of different samples of: (*a*) *Madhuca indica*, (*b*) *Malinkara zapota*, and

230 (*c*) *Mimusops elengi*.

231 **Table 2.** Main absorption bands in the ATR-FTIR spectra for leaves, seed coat and seed
232 kernel samples from three species of the *Sapotaceae* family (all wavenumbers are
233 expressed in cm^{-1})

M. indica leaves	*M. zapota* leaves	*M. elengi* leaves	*M. indica* seed coat	*M. zapota* seed coat	*M. elengi* seed coat	*M. indica* seed kernel	*M. zapota* seed kernel	*M. elengi* seed kernel
3331	3333	3336	3340	3360	3340	3293	3296	3301
2919	2917	2918	2919	2923	2920	2917	2922	2922
	2850	2850	2851	2854	-	2850	2853	2853
1733	1733	1732	1733	1733	1731	1743	1744	1744
1597	1614	1617	1606	1596	1601	1634	1652	1661
-	-	-	-	-	-	1538	1547	1547
1505	1507	1506	1504	1506	1507	-	-	-
1451	1446	1452	1444	1454		1455	1456	1455
1423	1424	1421		1422	1429	1416	1416	1416
1369	1367	1368	1373	1371	1370	1378	1377	1377
1318	1317	1318	1336	1326	1320	1316	1316	1316
1234	1241	1236	1236	1232	1236	1239	1239	1239
1153	1147	1154	1155	1157	1158	1158	1143	1136
1031	1031	1032	1034	1031	1032	1035	1034	1038
896	897	894	881	895	896	923	1000	926
819	826	828	824	825	823	831	831	831
779	769	780	729	764	776	717	720	776
557	558	557	549	557	558	557	-	568

234

Analysis of band maxima positions. The absorption bands at 3330 cm^{-1} in the seed coat samples were shifted 30 cm^{-1} towards higher wavenumbers as compared to the kernel and leaves samples. Another is the case for the absorption bands at 896-881 cm^{-1}, which were shifted 30 cm^{-1} towards lower wavenumbers than in kernel samples or, as it occurred for the band at 1740 cm^{-1}, were shifted between 3 and 11 cm^{-1} towards lower wavenumbers than in the kernel samples. For leaves samples the band that occurred at 1318 cm^{-1} was shifted towards higher wavenumbers *vs*. those in seed coat samples and towards lower wavenumbers *vs*. seed kernel ones, whereas the 1043 cm^{-1} band was shifted to higher wavenumbers in comparison with the seed coat and seed kernel samples (although this shift was more pronounced for seed coat samples). The band at 560 cm^{-1} was absent in seed kernel samples.

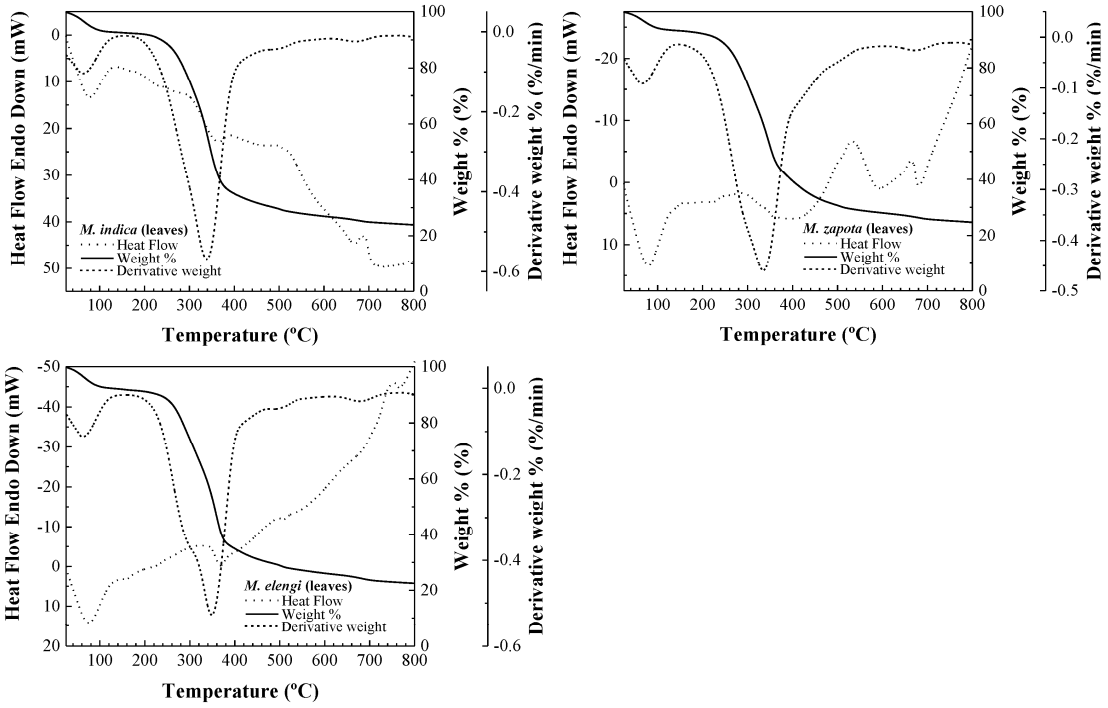
Analysis of absorbance intensities. For all the seed kernel samples, a noticeable increase in intensity occurred for the bands at 2920 cm^{-1}, 2850 cm^{-1} and 1733 cm^{-1}, whereas a decrease in intensity occurred for the band at 1232 cm^{-1}. For *M. indica* seed coat sample, an increase in intensity was found for the bands at 1450 cm^{-1} and 820 cm^{-1}.

Comparison with spectra from leaves extracts. FTIR bands in the crude *M. elengi* leaves extracts recorded by Prakashet et al. (**29**) appeared at 2928 cm^{-1}, 1618 cm^{-1}, 1445 cm^{-1} and 1041 cm^{-1}, in excellent agreement to those reported above for leaves and seeds.

3.3. Thermal Characteristics

DSC, DTG and TG curves were registered for the nine samples under study (**Figs. 4-6**). **Fig. 7** shows a comparison of the TG curves for *M. indica*, *M. zapota* and *M. elengi*

257 seed samples, evidencing differences in weight loss. Leaves and seed coat samples
258 curves were distinguishable from those of seed kernel samples because the curves of the
259 former two showed a plateau of stability between 100 ^{0}C and 300 ^{0}C, whereas in kernel
260 samples there was a continuous loss of mass from the beginning up to 240 ^{0}C. On the
261 basis of the temperature of the endothermic effects above 300 ^{0}C, it can be deduced
262 that, at higher temperatures, seed kernel samples were more stable (400 ^{0}C) than leaves
263 and seed coat samples. Furthermore, *M. elengi* seed coat samples showed higher
264 stability than those of *M. indica* and *M. zapota*.
265



266

267 **Fig. 4.** DSC (*dotted line*, y-axis on the left side of the graph), TG (*solid line*, first y-axis
268 on the right side of the graph) and DTG (*dashed line*, second (rightmost) y-axis on the
269 right side of the graph) curves for *M. indica*, *M. zapota* and *M. elengi* leaves samples.

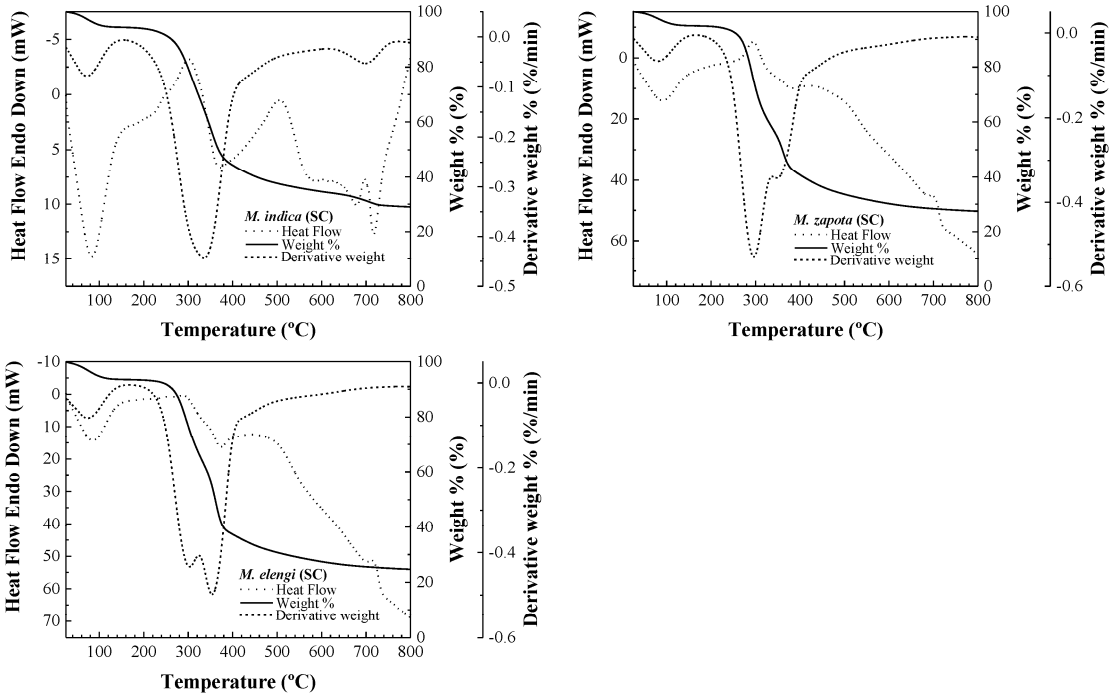
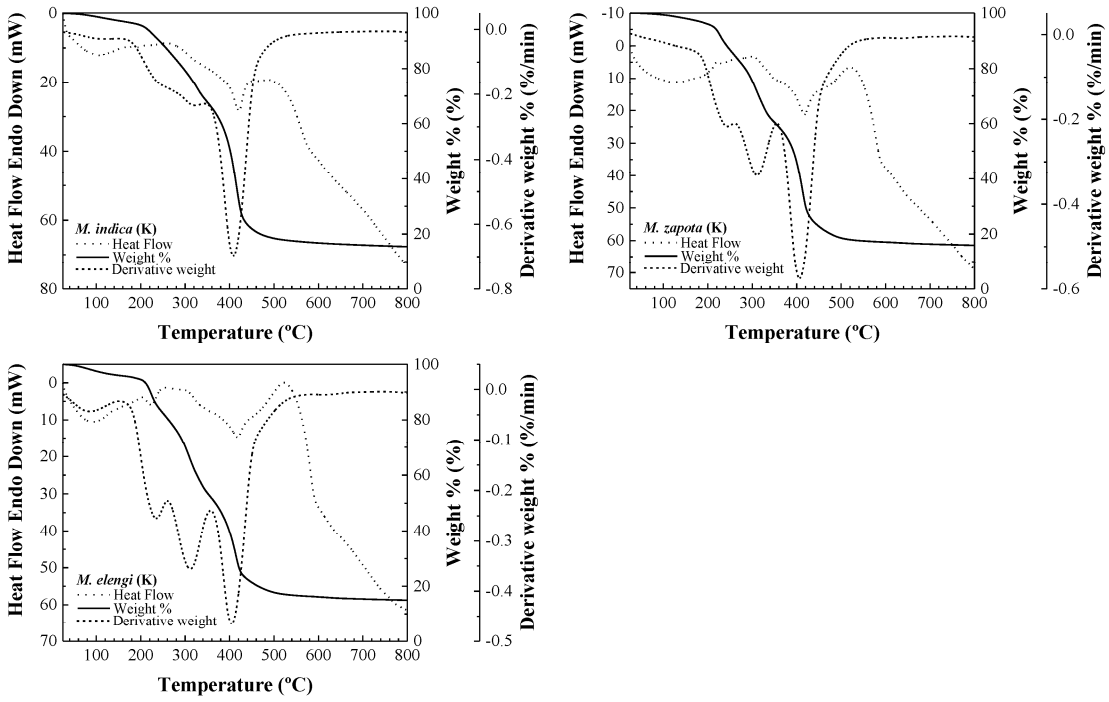


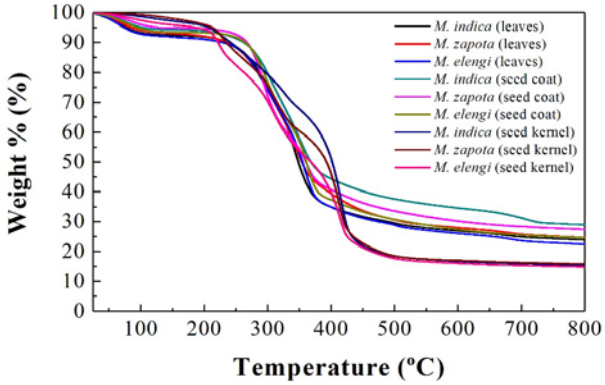
Fig. 5. DSC (*dotted line*, y-axis on the left side of the graph), TG (*solid line*, first y-axis on the right side of the graph) and DTG (*dashed line*, second (rightmost) y-axis on the right side of the graph) curves for *M. indica, M. zapota* and *M. elengi* seed coat samples.



277

278 **Fig. 6.** DSC (*dotted line*, y-axis on the left side of the graph), TG (*solid line*, first y-axis
279 on the right side of the graph) and DTG (*dashed line*, second (rightmost) y-axis on the
280 right side of the graph) curves for *M. indica, M. zapota* and *M. elengi* seed kernel
281 samples.

282



283

284 **Figure 7.** Comparison of the TG curves for *M. indica, M. zapota* and *M. elengi* seed
285 samples evidencing differences in weight loss.

286

287 **3.4. Oil and Starch Contents**

288 The phytochemical characteristics of seeds and leaves are shown in **Table 3**. The oil
289 fraction in the seed kernel MI, MZ and ME was found to be 54.1±1.1%, 25.2±0.5% and
290 9.8±0.2%, respectively. The insoluble (resistant), soluble and total starch contents in the
291 kernel of MI, MZ and ME ranged from 0.81 to 2.29%, from 6.7 to 9.3%, and from 7.51
292 to 11.59%, respectively. The maximum oil, resistant and soluble starch contents were
293 found in the MI seed kernel.

294 Taking caloric values of 9, 4 and 2 kcal per gram of oil, protein and starch,
295 respectively [30], the estimated caloric value for 100 g (dw) of MI, MZ and ME seed
296 kernel would be *ca*. 647, 507 and 434 kcal, respectively.

297

298 **Table 3**. Phytochemical characteristics of plant parts from the three Sapotaceae species.

Parameter	*Madhuca indica*			*Manilkara zapota*			*Mimusops elengi*		
	Leaves	Seed kernel	Seed coat	Leaves	Seed kernel	Seed coat	Leaves	Seed kernel	Seed coat
TPh	25900±510 a	23180±466 a	7810±154 a	24260±485 b	1850±38 b	11140±21 b	28600±510 c	2100±43 b	23060±432 c
Fla	19480±380 a	11700±234 a	1980±37 a	15870±304 b	13900±270 b	2050±38 a	11110±228 c	2700±46 c	5870±120 b
TPh/Fla	1.3	2.0	3.9	1.5	0.1	5.4	2.6	0.8	3.9
Oil, %	-	54.1±1.1 a	-	-	25.2±0.5 b	-	-	9.8±0.2 c	-
Soluble Starch, %	-	9.3±0.2 a	-	-	8.5±0.2 b	-	-	6.7±0.1 c	-
Resistant starch, %	-	2.30±0.05 a	-	-	0.77±0.02 b	-	-	0.81±0.03 b	-

TPh = total phenolic content; Fla = flavonoid content. The contents of the various constituents in the same plant part (either leaves, seed kernel or seed coat) labelled with the same lowercase letters were not significantly different at $p < 0.05$ using Tukey's test.

3.5. Phenol Content

The phenol contents of MI, MZ and ME leaves, kernel and seed coat are summarized in **Table 3**. Polyphenols are secondary metabolites of plants, generally involved in defense against ultraviolet radiation or aggression by pathogens. Among them, flavonoids are the most well-known active polyphenols. The total polyphenolic (TPh) content in the leaves, seed kernel and seed coat of MI, MZ and ME ranged from 24260 to 28600 mg/kg, from 1850 to 23180 mg/kg, and from 7810 to 23060 mg/kg as tannic acid equivalents, respectively (**Table 3**). Similarly, flavonoid (Fla) contents in the leaves, seed kernel and seed coat of MI, MZ and ME varied from 11110 to 19480 mg/kg, from 2700 to 13900 mg/kg, and from 1980 to 5870 mg/kg as quercetin equivalents, respectively (**Table 3**). A very high TPh content (> 20000 mg/kg) was detected in the leaves and seed kernel from MI, in the leaves from MZ, and in the leaves and seed coat from ME, respectively. In same way, high Fla contents (> 11000 mg/kg) were found in leaves from MI, MZ and ME. The {[TPh]/[Fla]} ratio in the leaves, seed kernel and seed coat of MI, MZ and ME varied from 1.3 to 2.6, from 0.1 to 2.0, and from 3.9 to 5.4, respectively (**Table 3**). A high {[TPh]/[Fla]} ratio, ≥3.9, was observed for the seed coats. It is worth noting that the lowest {[TPh]/[Fla]} ratio was recorded for MZ seed kernel.

3.6. Mineral Contents

The sum of the total concentrations of 19 elements (viz. K, Rb, Mg, Ca, Sr, Al, P, S, Cl, As, Ti, V, Mn, Fe, Co, Cu, Zn, Mo and Pb) in the leaves, seed kernel and seed coat from MI, MZ and ME varied from 882 to 26763 mg/kg, from 11390 to 19385 mg/kg, and from 3619 to 14963 mg/kg, respectively (**Table 4**). Six nutrients, viz. P, S, Ca, Fe, Cu and Rb, were detected in all leaves, seed kernel and seed coat samples. A remarkably high concentration of P was identified in the seed kernel of the three species, ranging from 871 to 1899 mg/kg. Extremely high concentrations of three elements (S, Ca and Fe) were detected in the leaves from MZ and ME. However, a different trend was observed for MI, in which the highest concentrations of S, Ca and Fe occurred in the seed coat. A high concentration of Cu was detected in the seed coats and leaves from MI and ME. Zn micronutrient was detected at moderate levels only in their kernels, ranging from 16 to 17 mg/kg. Molybdenum was detected at low levels (3 mg/kg) in ME leaves. Toxic elements, such as As and Sr, were identified in all leaves at very low levels, ranging from 1.0 to 2.0 mg/kg and from 4 to 46 mg/kg, respectively. It is worth noting that the very toxic Pb was identified at moderate levels (10–19 mg/kg) in ME leaves and seed kernel, i.e., at concentrations several folds higher than the permissible exposure limit of 0.3 mg/kg (**31**).

The seed cake is the product resulting after extraction of oil from the kernel. Since MI, MZ and ME seed kernels contained 54.1%, 25.2% and 9.8% oil, respectively, the seed cakes from MI, MZ and ME would be enriched by a factor of 2.17, 1.33 and 1.11 in terms of starch, protein, phenols and mineral contents. The enriched concentration of

starch in the seed cakes from MI, MZ and ME would be 25.2%, 12.3% and 8.3%, respectively. Concentrations of TPh, Fla and TM (total mineral) in the seed cakes from MI, MZ and ME were 48803, 9722, 29056 mg TPh/kg; 2386, 7129, 24109 mg Fla/kg; and 2251, 977 and 21517 mg/kg, respectively. As expected, the seed cake of MI was the most enriched, featuring the highest contents in minerals and phenols. However, a remarkably high {[TPh]/[Fla]}ratio of 22 was observed for the ME cake.

Table 4. Mineral contents in the plant parts from the three selected Sapotaceae species.

Parameter	*Madhuca indica*			*Manilkara zapota*			*Mimusops elengi*		
	Leaves	Seed kernel	Seed coat	Leaves	Seed kernel	Seed coat	Leaves	Seed kernel	Seed coat
Mg	<DL	<DL	921	1345	611	<DL	1408	670	<DL
Al	<DL	<DL	<DL	153	<DL	<DL	381	<DL	<DL
P	475	1293	650	612	1899	56	630	871	320
S	439	1083	1382	2334	1879	250	2357	1658	479
Cl	1705	37	1704	1896	<DL	<DL	940	1923	<DL
K	4872	10643	8987	8875	8816	<DL	3233	8300	2108
Ca	1104	266	1750	8994	4803	5975	16256	5822	617
Ti	<DL	<DL	<DL	93	<DL	<DL	38	<DL	<DL
V	<DL	<DL	<DL	1.5	<DL	<DL	1.5	<DL	<DL
Mn	17	<DL	29	153	8	13	60	25	<DL
Fe	182	29	235	2912	47	125	1261	68	76
Co	<DL	1	<DL	2.5	1	1	4	1	1
Cu	13	4	214	45	5	6	123	3	5
Zn	<DL	16.5	<DL	<DL	16.5	<DL	<DL	15.5	2.5
As	1.5	<DL	<DL	2	<DL	<DL	1	<DL	<DL

Rb	11	17	9.5	9	23.5	1	1.5	9	6
Sr	3	<DL	2.5	34.5	18	29	46	10	4
Mo	<DL	<DL	<DL	<DL	<DL	<DL	2.5	<DL	<DL
Pb	<DL	<DL	<DL	<DL	<DL	<DL	19	9.5	<DL

<DL = Below detection limit

3.7. Correlation Coefficients

Correlations among oil, starch, TPh, Fla and mineral element contents in the seed kernels from the three selected Sapotaceae trees are summarized in **Table 5**. Phosphorous showed a good correlation with Fla, K, Cu, Zn and Rb (r = 0.81–0.99), in agreement with P-micronutrient interactions observed in other species (**32**), mainly for P-Zn when available K levels are increased (**33**), and also in agreement with studies that have shown that Rb is absorbed via a carrier that also applies to K (**34**). Sulphur exhibited fair/strong (r = 0.67–0.98) correlations with a series of elements (viz. Mg, Ca, Sr and Fe), suggesting their accumulation as sulfide or sulfate compounds. Strong statistical correlations were also found among oil, starch, TPh, K and Zn; Mg, Ca, Sr, Mn and Fe; and Cl, Mn, Fe and Pb, indicating their accumulation as cofactor elements.

While oil and TPh contents in the seed kernels showed a positive correlation with the seed kernel mass (r = 0.93 - 0.95), a reverse trend (r = -0.98) of the total element content with the seed kernel mass was found. Both for seed coat and leaves, the Fla content was statistically correlated with the mass (r = 0.94 and r=0.63, respectively). In the leaves, arsenic showed a moderate to strong correlation with TPh and Fla content (r = 0.63-0.93), possibly due to complex formation.

Table 5. Correlation coefficient matrix for the different constituents found in the Sapotaceae seed kernels.

	Oil	Starch	TPh	Fla	Mg	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Rb	Sr	Pb
Oil	1.00																
Starch	1.00	1.00															
TPh	0.94	0.90	1.00														
Fla	0.63	0.70	0.32	1.00													
Mg	-0.96	-0.93	-1.00	-0.40	1.00												
P	0.24	0.33	-0.11	0.90	0.02	1.00											
S	-0.81	-0.75	-0.97	-0.06	0.94	0.37	1.00										
Cl	-0.76	-0.81	-0.48	-0.99	0.55	-0.82	0.23	1.00									
K	0.99	0.97	0.98	0.52	-0.99	0.11	-0.89	-0.66	1.00								
Ca	-0.98	-0.96	-0.98	-0.49	1.00	-0.07	0.90	0.63	-1.00	1.00							
Mn	-0.93	-0.96	-0.74	-0.88	0.80	-0.59	0.54	0.94	-0.87	0.85	1.00						
Fe	-0.98	-0.99	-0.84	-0.79	0.88	-0.45	0.67	0.88	-0.94	0.92	0.99	1.00					
Cu	0.34	0.43	-0.01	0.94	-0.08	0.99	0.27	-0.87	0.21	-0.17	-0.67	-0.54	1.00				
Zn	0.77	0.82	0.49	0.98	-0.57	0.81	-0.25	-1.00	0.67	-0.64	-0.95	-0.89	0.87	1.00			
Rb	0.40	0.48	0.05	0.96	-0.14	0.99	0.21	-0.90	0.27	-0.23	-0.71	-0.59	1.00	0.89	1.00		
Sr	-0.69	-0.62	-0.90	0.12	0.86	0.53	0.98	0.05	-0.78	0.81	0.37	0.52	0.44	-0.06	0.39	1.00	
Pb	-0.77	-0.82	-0.49	-0.98	0.57	-0.81	0.25	1.00	-0.67	0.64	0.95	0.89	-0.87	-1.00	-0.89	0.06	1.00

TPh = Total phenolic, Fla = Flavonoid

4. CONCLUSIONS

The seeds from the three selected Sapotaceae trees featured high lipid contents, in the 9.8–54.1% range, as well as moderate starch concentrations, in the 6.7–9.3% range, in

good agreement with the information retrieved from their FTIR spectra. The leaves and seed cakes from *M. indica* and *M. zapota* were found to be promising sources of nutrients and antioxidants, including polyphenols, flavonoids, P, S, K, Ca and Fe, suggesting a possible valorization in animal feeding and as herbal medicines. The concentrations of toxic elements, such As and Sr, remained below safety limits. Another is the case for *M. elengi* leaves and seed kernel, in which Pb concentration was higher than the allowed exposure limit, precluding its use for aforementioned applications. In view of the thermal stability shown by its samples, *M. elengi* biomass could be valorized as a raw material for production of thermoplastics or for biodiesel production, due to the robust combustion characteristics of its seed kernels.

CONSENT

Not applicable.

ETHICS APPROVAL

Not applicable.

CONFLICT OF INTEREST

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

REFERENCES

1. Chanda SV, Nagani KV. Antioxidant capacity of *Manilkara zapota* L. leaves extracts evaluated by four *in vitro* methods. Nature and Science. 2010;8(10):260-266.

2. Kadam PV, Yadav KN, Deoda RS, Shivatare RS, Patil MJ. *Mimusops elengi*: A review on ethnobotany, phytochemical and pharmacological profile. Journal of Pharmacognosy and Phytochemistry. 2012;1(3):64-74.

3. Khare CP. Encyclopedia of Indian medicinal plants. Springer Publication. (2004) 314-315.

4. Mehnaz B, Bilal A. *Manilkara zapota* (L.) P. Royen (Sapodilla): A review. International Journal of Advance Research, Ideas and Innovations in Technology. 2017;3(6):1364-1371.

5. Sangeetha R, Devi N. *Madhuca longifolia* (Sapotaceae): A review of its phytochemical and pharmacological profile. International Journal of Pharma and Bio Sciences. 2016;7(4):106–114. doi: 10.22376/ijpbs.2016.7.4.b106-114.

6. Sharma P, Chaturvedi N, Upadhyay M, Varma S. Quantitative determination of total phenolic content in stem bark and leaves extracts of *Madhuca longifolia*. International Journal of Pharm Tech Research. 2013;5(3):1150-1154.

7. Singh A, Singh IS. Chemical evaluation of Mahua (*Madhuca Indica*) seed. Food chemistry. 1990;40(2):221-228.

8. Mishra S, Sarojini P. *Madhuca longifolia* (Sapotaceae): a review of its traditional uses and nutritional properties. International Journal of Humanities and Social Science Invention. 2013;2:30-36.

9. Yadav P. *Madhuca longifolia* (Sapotaceae): a review of its traditional uses, phytochemistry and pharmacology. International Journal of Biomedical Research. 2012;3(7):201-305. doi:10.7439/ijbr.v3i7.292.

10. Jha D, Mazumder PM. Biological, chemical and pharmacological aspects of *Madhuca longifolia*. Asian Pacific Journal of Tropical Medicine. 2018;11(1):9-14. doi: 10.4103/1995-7645.223528.
11. Fayek NM. Chemical and biological study of *Manilkara zapota* (L.) Van Royen leaves (Sapotaceae) cultivated in Egypt. Pharmacognosy Research. 2012;4(2):85–91. doi: 10.4103/0974-8490.94723.
12. Baliga MS, Pai RJ, Bhat HP, Palatty PL, Bolo R. Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn): A review. Food Research International. 2011;44(7):1823-1829, doi: 10.1016/j.foodres.2011.01.063.
13. Ghadge S, Raheman H. Biodiesel production from Mahua *(Madhuca indica)* oil having high free fatty acids. Biomass and Bioenergy. 2005;28(6):601-605. doi: 10.1016/j.biombioe.2004.11.009.
14. Kumar RS, Sureshkumar R, Velraj K. Optimization of biodiesel production form *Manilkara zapota* (L.) seed oil using Taguchi method. Fuel. 2015;140:90–96. doi:10.1016/j.fuel.2014.09.103.
15. Kalaiselvi V, Binu TV, Radha SR, Vijayakumari B. Antioxidant activity of various leaf extracts of *Mimusops elengi* L. International Journal of Pharmacy and Pharmaceutical Research. 2015;3(4):231-237.
16. Shanmugapriya K, Saravana PS, Payal H, Mohammed SP, Binnie W. Antioxidant activity, total phenolic and flavonoid contents of *Artocarpus heterophyllus* and *Manilkara zapota* seeds and its reduction potential. International Journal of Pharmacy and Pharmaceutical Research. 2011;3(5):256-260.

17. Shahwar D, Raza MA. Antioxidant potential of phenolic extracts of *Mimusops elengi*. Asian Pacific Journal of Tropical Biomedicine. 2012;2(7):547-550.
18. Uekane TM, Nicolotti L, Griglione A, Bizzo HR, Rubiolo P, Bicchi C, Helena M, Rocha-Leão M, Rezende CM. Studies on the volatile fraction composition of three native Amazonian-Brazilian fruits: Murici (*Byrsonima crassifolia* L., Malpighiaceae), bacuri (*Platonia insignis* M., Clusiaceae), and sapodilla (*Manilkara sapota* L., Sapotaceae). Food chemistry. 2017;219:13-22. doi: 10.1016/j.foodchem.2016.09.098.
19. Khare CP. Indian Medicinal Plants. Springer-Verlag New York, 2007.
20. Singh KK, Goswami TK. Physical properties of Cumin seed. Journal of Agricultural Engineering Research. 1996;64:93-98. doi: 10.1006/jaer.1996.0049.
21. Gornas P, Rudzinska M, Segliņa D. Lipophilic composition of eleven apple seed oils: A promising source of unconventional oil from industry by-products. Industrial Crops & Products. 2014;60:86–91. doi: 10.1016/j.indcrop.2014.06.003.
22. AOAC Official Method, Resistant starch in starch and plant materials enzymatic Digestion First Action, 2002.
23. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substances by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999;299:152-178. doi: 10.1016/S0076-6879(99)99017-1.
24. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by complementary colorimetric methods. Journal of Food and Drug Analysis. 2002;10:178-182.

462 25. Fengel D, Ludwig M. Moglichkeiten und Grenzen der FTIR-Spektroskopiebei der
463 Charakterisierung von Cellulose. I: Vergleich von Verschiedenen Cellulosefasern
464 und Bakterien-Cellulose. Das Papier. 1991;45:45-51.
465 26. Wang XQ, Zhou RW, Groot DG, Bazaka K, Murphy AB, Ostrikov K. Spectral
466 characteristics of cotton seeds treated by a dielectric barrier discharge plasma.
467 Scientific Reports. 2017;7:5601. doi: 10.1038/s41598-017-04963-4.
468 27. Singh S, Bothara SB. *Manilkarazapota* (Linn.) seeds: A potential source of natural
469 gum. International Scholarly Research Notices Pharmaceutics. 2014;647174:1-10.
470 doi: 10.1155/2014/647174.
471 28. Martín-Ramos P, Carrión-Prieto P, Ruiz-Potosme NM, Hernández-Navarro S,
472 Martín-Gil J. An analysis of the similarities in the ATR-FTIR spectra from *Argania*
473 *spinosa*, *Rosa rubiginosa* and *Elaeis guineensis* oils, Journal of Essential Oil
474 Bearing Plants. 2017;20(6):1651-1658, doi: 10.1080/0972060X.2017.1396926
475 29. Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S, Saravanan M. Green
476 synthesis of silver nanoparticles from leaf extract of *Mimusops elengi* Linn. for
477 enhanced antibacterial activity against multidrug resistant clinical isolates. Colloids
478 and Surfaces B: Biointerfaces. 2013;108:255-259.
479 30. Meiners CR, Derise NL, Lau HC, Crews MG, Ritchey SJ, Murphy EW. Proximate
480 composition and yield of raw and cooked mature dry legumes. J. Agric. Food
481 Chem. 1976;24(6):1122-1126.
482 31. FAO/WHO, Joint FAO/WHO Food Standards Programme Codex Committee on
483 Contaminants in Foods Food. CF/5 INF/1, 2011, pp. 1.

32. Hopkins BG, Rosen CJ, Shiffler AK, Taysom TW. Enhanced efficiency fertilizers for improved nutrient management: Potato (*Solanum tuberosum*). Crop Management. 2008;**7**:1-16. doi: 10.1094/CM-2008-0317-01-RV

33. Daliparthy J, Barker AV, Mondal SS. Potassium fractions with other nutrients in crops: A review focusing on the tropics. Journal of Plant Nutrition, 1994;17(11):1859-1886. doi.org/10.1080/01904169409364852)

34. Epstein E, Hendricks SB. Uptake and transport of mineral nutrients in plant roots. Proceedings International Conference Peaceful Uses of Atomic Energy (United Nations). 1956;12:98-102.