Original Research Article

Nutritional and Spectral Characteristics of *Terminalia* Plants

ABSTRACT

Aims*: Terminalia* are medicinal plants belong to family *Combretaceae* widely used in the traditional Ayurvedic medicines. In this work, the nutritional constituents of leaves, seed kernel and seed coat from four *Terminalia* species (*T. arjuna*, *T. bellirica*, *T. catappa* and *T. chebula*) are reported.

Methodology: The polyphenol and flavonoid content of the *Terminalia* species were analyzed spectrophotometrically by using Folin-Ciocalteu and aluminum chloride as reagent, respectively. The mineral contents were quantified by using X-ray fluorescence (XRF) technique by using reference materials. The functional groups of the phytochemicals were assessed by the FTIR technique.

Results: The total concentrations of 20 macro- and micronutrients (viz. P, S, Cl, K, Rb, Mg, Ca, Sr, Ba, Al, Ti, Cr, Mn, Fe, Co, Cu, Zn, Mo, As and Pb), total polyphenol and flavonoid contents in four seed kernels ranged from 1453 to 65461 mg/kg, from 2150 to 51100 mg/kg and from 63 to 42300 mg/kg, respectively. Similarly, the polyphenol and mineral content for the *Terminalia* seed coats and leaves are described. The enrichment of the nutrient with respect to the soil content in the *Terminalia* plants are discussed. The vibrational spectra from leaves and seed coats agree with a composition rich in lignin, hemicellulose, cutin, pectin and flavonoids, while seed kernels are in accordance with a greater presence of unsaturated oils, protein, and fiber.

Conclusion: Various parts of all investigated *Terminalia* species (*T. arjuna*, *T. bellirica*, *T. catappa* and *T. chebula*) are enriched with high content of nutrients and polyphenols which are needed for biological metabolism and human health. In addition, heavy metals are present in the traces which shows *Terminalia* plants safe for medicinal uses.

Keywords: *Terminalia*; FTIR; XRF; flavonoid; phenolic; nutrients.

1. Introduction

Terminalia genus comprises around 100 species distributed in tropical regions of the world. Trees of this genus, common in plains and low hills in India, are especially known as a source of secondary metabolites, such as tannins, cyclic triterpenes and their derivatives, flavonoids, and other aromatics. Tannin-containing cells occur throughout in the plant body, particularly in the pericarp of the fruit.

Terminalia species are important medicinal plants: they are administered as astringent and purgative, and are used in dropsy, diarrhea, piles, leprosy, and cough treatments [**1**]. The phytochemical and pharmacological profile of *Terminali arjuna* (Roxb. ex DC.) Wight & Arn., known as Arjun, has been reported in the review paper by Jain *et al*. [**2**]. *T. bellirica* (Gaertn.) Roxb., known as Bahera or Beleric, and *Terminalia chebula* Retz., known as Chebulic myrobalan, are two main constituents of Triphala, traditionally used to treat various gastrointestinal disorders [**3**], and an evaluation of the pharmacological activities of the latter has been covered in a review paper by Bag *et al*. [**4**]. The phytoconstituents and pharmacological benefits of *Terminalia catappa* L., known as Indian-Almond, have been discussed in the review paper by Anand *et al*. (**5**).

The antioxidant, antifungal and antibacterial properties of some species of *Terminalia* have been reported in the literature [**6-10**] and there are data on the volatile compounds identified in the fruits and essential oils from *T. arjuna*, *T. catappa* [**11-16**] and *T. chebula* [**17**], but most nutritional-related information remains unreported. In this work, a comparative study of the nutritional (i.e., polyphenolic and trace elements) content of the leaves, seed kernel and seed coat from the four *Terminalia* species mentioned above (*T. arjuna*, *T. bellirica*, *T. catappa* and *T. chebula*) are presented.

2. METHODS AND MATERIALS

2.1. Sampling of Plants

The four *Terminalia* species discussed herein (viz. *T. arjuna* (TA), *T. bellirica* (TB), *T. catappa* (TC) and *T. chebula* (TCh)) grow massively in the Chhattisgarh region, in the in the center-east of India. They were botanically authenticated with the aid of standard monographs [**18**]. The leaves and fruits of TA and TB were collected in May 2017 from Raipur city (21.25°N 81.63°E), while the leaves and fruits of TC and TCh were collected in December 2017. The near-surface layer of the soils was also sampled.

2.2. Sample Preparation

The leaves, pericarp, seed coat and seed kernel were manually separated. First, they were cleaned with the de-ionized water and dried with the hot air blower. They were sundried in a glass room for one week, further dried for 24 hr at 50 ^{0}C in a hot air oven, and were finally stored in glass containers. The plant and soil samples were crushed into fine powder with a mortar and sieved out particles of mesh size of ≤ 100 μm. They were preserved at -4 ^{0}C in the deep freezer.

2.3. Analysis

Sample weights were measured with a Mettler-Toledo (Columbus, OH, USA) electronic balance. The moisture content of the samples was determined by drying at 105 ^{0}C in an air oven for 6 hr prior to the analysis. All characterization results are presented on a dry weight (dw) basis.

The total phenolic content (TPC) and flavonoid content (Fla) were determined as follows: firstly, 100 mg of powered sample was dispersed in 5 mL of an acetone:water (70:30, v/v) solution, which was sonicated in an ultrasonic bath for 20 min at 20 ^{0}C. Then, 5 mL of fresh acetone:water (70:30, v/v) solution was 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 equivalent (TAE) by using the Folin-Ciocalteu reagent [**19**]. The flavonoid content was determined by the aluminum chloride method as quercetin equivalents (QE) [**20**].

For macro- and micronutrient analyses, X-ray fluorescence (XRF) technique was chosen, using a Bruker III Tracer SD (T3S2731 (Kennewick, WA, USA) spectrometer equipped with a 4W rhodium anode and Xflash SDD 2028 channels. Standard brown and white cowpea (*Vigna unguiculata* (L.) Walp.) seeds and soil sample ((NCS DC 73382 CRM) were used for calibration.

The Fourier-Transform Infrared (FTIR) spectra were characterized with a Thermo Scientific (Waltham, MA, USA) Nicolet iS50 spectrometer equipped with an in-built diamond attenuated total reflection (ATR) system. Spectra were collected in the 400-4000 cm^{-1} spectral range, with a 1 cm^{-1} spectral resolution and averaging 64 scans. All analyses were carried out in triplicate, and mean values are reported.

3. RESULTS AND DISCUSSION

3.1. Plant Characteristics

The physical characteristics of the leaves and seeds from the four *Terminalia* species are shown in **Table 1**. The leaves, seeds and seed kernels were colored, with various shapes, as shown in **Figure 1**. Micrographs of leaves samples are shown in **Figure 2**. The average mass of a single leaf of TA, TB, TC and TCh was 2367±41, 3700±66, 7500±142 and 3767±67 mg, respectively. The mass of a single seed on dry weight basis was 3885±75, 4373±81, 4762±78 and 5426±102 mg, with a kernel fraction of 3.1, 11.0, 8.3 and 2.1%, respectively (i.e., the seed coats are hard and thick and accounted for a remarkably high fraction of the seed weight). The water content in the leaves, seed coat and seed kernel were ranged from 2.8–4.9%.

3.2. Phenolic content

The phenolic content for the four *Terminalia* species is shown in **Table 1**, with TPC values in the leaves, seed coat and seed kernel ranging from 23900 to 33100 m/kg, from 22400 to 51100 mg/kg and from 2150 to 9530 mg/kg, respectively. Similarly, Fla concentration in the leaves, seed coat and seed kernel varied in the 11200–25900 mg/kg, 5300–42300 mg/kg and 63–2150 mg/kg range, respectively. Plant parts from TCh were found to contain the highest contents of TPC and Fla. The Fla/TPC ratio in the leaves, seed coat and seed kernel showed mean values of 0.69, 0.54 and 0.11, respectively.

3.2. Macro- and Micronutrients Content

The mineral element concentrations are presented in **Table 2**. As regards macronutrients, which play a major role in plant physiological processes, P concentration in leaves, seed coat and seed kernel ranged from 51 to 772 mg/kg, from 287 to 1109 mg/kg, and from 3842 to 8171 mg/kg, respectively. Relatively higher concentrations of K were detected, which varied in the 288–9364 mg/kg, 3683–16001 mg/kg, and 4334–13947 mg/kg range in the leaves, seed coats and kernels, respectively. Rubidium which has chemical properties similar to K^+ in the biological processes [**22**], showed concentrations in the leaves, seed coats and kernels of 1–16 mg/kg, 9–25 mg/kg, and 13–28 mg/kg, respectively.

Apropos of the secondary macronutrients (viz. S, Mg and Ca), the concentrations of S in the leaves, seed coat and kernel were in the 71–606 mg/kg, 176-545 mg/kg, and 1166–3158 mg/kg intervals, respectively. Magnesium concentrations in the leaves, seed coat and kernel varied from 105 to 1868 mg/kg and 11 to 1316 mg/kg, and from 828 to 5440 mg/kg, respectively. Calcium concentrations in the leaves, seed coat and kernel were in the 919–49656 mg/kg, 699–6644 mg/kg and 2031–9443 mg/kg ranges, respectively. Strontium showed concentrations in the range of 3-101, 2-20 and 2-28 mg/kg for leaves, seed coat and seed kernel, respectively. Barium was detected in the leaves, seed coat and kernel between 1-39, 1-2 and 1-12 mg/kg, respectively.

Chloride was detected only in the leaves and seed coat of all *Terminalia* species, ranging from 46-3346 and 173-3287 mg/kg, respectively.

Titanium, which stimulates enzyme activities and uptake of nutrients [**23**], was detected only in the TA kernel and TCh seed coat at low levels, 15 and 42 mg/kg, respectively.

Chromium was identified in the leaves and seed coats of all species between 1-7 and 1-37 mg/kg, respectively.

Manganese, necessary in photosynthesis and nitrogen metabolism, was identified in all parts of the *Terminalia* species which varied from 14-66, 3-63 and 17-88 mg/kg for the leaves, seed coats and kernels, respectively.

Iron, involved in production of chlorophyll, lignin formation, etc., was detected at moderate to high levels, varying from 127-229, 100-937 and 71-140 for the leaves, seed coats and kernels, respectively.

Cobalt, an essential component of several enzymes, was detected at low levels, 1-6 mg/kg in all parts of the Terminalia species.

Copper, necessary for carbohydrate and nitrogen metabolism, was detected in the leaves, seed coats and kernels of all *Terminalia* species, ranging from 1-15, 3-771 and 17-38 mg/kg.

Zinc –an essential component of various enzyme systems for energy production, protein synthesis, and growth regulation– was identified in the leaves, seed coats and kernels, varying from 3-7, 1-5 and 22-59 mg/kg, respectively.

Molybdenum, involved in enzyme systems relating to nitrogen fixation by bacteria, was found at low concentration in the leaves and seed coats, ranging from 1-4 and 2–20 mg/kg. It was only detected in the leaves of TA and CA at low levels (2–4 mg/kg). Arsenic was detected in leaves at low levels, 1-2 mg/kg. Whereas, Pb concentration was varied relatively at higher level, 2-10 and 1-11 mg/kg in the leaves and seed coats.

The total concentration of 20 elements (i.e. P, S, Cl, K, Rb, Mg, Ca, Sr, Ba, Al, Ti, Cr, Mn, Fe, Co, Cu, Zn, Mo, As and Pb) in the leaves, seed coat and kernel of TA, TB, TC and TCh was 65521, 30832 and 40523; 5733, 6510 and 2493; 1754, 11634 and 12408; and 11009, 10254 and 24189 mg/kg, respectively. Remarkably high concentration of the elements in all parts of the TA was marked.

3.3. Soil Characteristics and Bioaccumulation Factor

In Chhattisgarh region, red laterites or entisols soil cover 19.5% of the cultivated area and yellow clayey inceptisol soil account for 14.8%, but in Raipur district the latter are the most frequent. These soils are slightly alkaline (mean value, 7.7; range 7.3–8.0), and show electrical conductivities (EC) in the range of 465–523 µS/cm, with mean value of 495 µS/cm (indicating an appreciable accumulation of salts).

The concentration in major and minor elements in the surface soil varied in the 114-141 mg/kg range for Cl (mean value, 127 mg/kg); 119–162 mg/kg for P (mean value, 138 mg/kg); 179–240 mg/kg for S (mean value, 207 mg/kg); 6.0–9.0 mg/kg for As (mean value, 7.5 mg/kg); 1339– 1510 mg/kg for K (mean value, 1438 mg/kg); 5.8–8.0 mg/kg for Rb (mean value, 6.9 mg/kg); 1450-1623 for Mg (mean value, 1545 mg/kg); 5880–6710 for Ca (mean value, 6304 mg/kg); 44–58 for Sr (mean value, 50 mg/kg); 29–45 for Ba (mean value, 37 mg/kg); 5460–7050 for Ti (mean value, 6412 mg/kg); 113–150 for Cr (mean value, 128 mg/kg); 1370–1660 for Mn (mean value, 1510 mg/kg); 17460–

20123 for Fe (mean value, 18818 mg/kg); 29–38 for Co (mean value, 32 mg/kg); 66–82 for Cu (mean value, 72 mg/kg); 73–96 for Zn (mean value, 87 mg/kg); 1.0–1.8 for Mo (mean value, 1.4 mg/kg) and 2–29 mg/kg for Pb (mean value, 15.5 mg/kg). They were found to occur in the following increasing order: Mo < Rb < As < Pb < Co < Ba < Sr < Cu < Zn < Cl ≈ Cr < P < S < K < Mn < Mg < Ca < Ti < Fe.

The K/P ratio (=10.4) was in good agreement with the ratio obtained from potassium and phosphorus values (=10.5) reported by Awanish et al. [**21**].

The bioaccumulation factor (BAF) is a ratio of the concentration of an element in the plant to the concentration of that element in soil, and depends on several factors, such as plant genotype, bioavailability of metals, soil quality, climatic condition, agronomic management, etc. BAFs are reported in **Table 3**. Several nutrients (K, P, Cl, S, Cl, Ca) were hyperaccumulated by the four *Terminalia* species, with the highest hyperaccumulation of K, P, Cl and S for *T. arjuna*.

Phenolic, macro- and micronutrients contents correlation matrix in seed kernel

The correlation coefficients of the elements for the Terminalia seed kernels are presented in **Table 4**. TPC showed a good correlation with the Fla P, S, Mg and Zn contents, exhibited high positive correlations with each other. Strong statistical correlations were found among elements i.e. P, S, K, Mg, Ca, Sr, Mn, Fe and Cu, indicating their accumulation as cofactor elements.

3.4. Vibrational characterization

The ATR-FTIR spectra for leaves, seed coat and seed kernel samples from the four species of the *Terminalia* genus under study are depicted in **Figure 3**. The corresponding bands are summarized in **Table 5**.

Peaks at around 3330 cm^{-1} (OH stretching) corresponded to typical characteristic absorption from cellulose [**24**]. Peaks at around 2920 cm^{-1} (–CH_2 aldehydic symmetrical stretching) and at 2853 cm^{-1} (-CH stretching) indicated the presence of cutine and wax. Peaks at *ca.* 1740 and at around 1370 cm^{-1} were indicative of hemicellulose, specifically of C=O stretching (1734 cm^{-1}) and -CH_3 symmetric deformation (1379-1362 cm^{-1}). Prominent bands in the 1340 to 890 cm^{-1} region were also attributed to cellulose: at 1336 cm^{-1} (δCH in-plane), at 1321-1311 cm^{-1} (C-H vibration), at around 1150 cm^{-1} (νC-O-C in bridge, asymmetric), at 1031-1027 cm^{-1} (νC-O or –C-O-C- stretching) and at *ca.* 896 cm^{-1} (νC-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 1457-1447 cm^{-1}) for calcium pectate and with –CH_3 distortion (1240-1229 cm^{-1}) for pectic ester. The band that appeared at around 1424 cm^{-1} can be attributed either to cellulose (ρCH_2, sym.) or to symmetric stretching vibration for calcium pectate (**25**). Bands at around 831 cm^{-1} were due to aromatic C-H out-of-plane binding or to C-O-C deformation and suggested the presence of D-Glc pyranoside configurations. Bands at 780 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 1618-1594 cm^{-1}; and the band of the aromatic skeletal vibration (C=C aromatic symmetrical stretching), at 1509-1505 cm^{-1}.

Seed kernel samples showed strong characteristic bands at around 1744 cm^{-1}, 1636 cm^{-1}, and 1540 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 around 1636 cm^{-1} could be characteristic of C=C absorption cellulose when it is cross-linked and dehydrated, but it may also be assigned to amide N–H & C=O stretching from mucilage [**26**] or to an enrichment in unsaturated oils. The presence of this band, typical of the vinyl group, would justify the quantitative presence of unsaturated oils in the kernel of all the seeds under study.

Analysis of band maxima positions. The absorption bands that occur at 3330 cm^{-1} for seed coat and leaves samples appeared shifted to 3380 cm^{-1} for kernel samples. The absorption band at 1723 cm^{-1} found in seed coats was shifted to 1743 cm^{-1} in seed kernels. As regards the band that occurred at 1053 cm^{-1} for kernel samples, shifts to 1031 cm^{-1} for seed coats and to 1027 cm^{-1} for leaves were observed. The band at 558 cm^{-1} was absent in seed kernel samples.

Results from the FT-IR spectra of leaves and seed coats showed that they are rich in lignin, hemicellulose, cutin, pectin and flavonoids, while unsaturated oils, protein, and fiber would be the main constituents of the seed kernels.

4. CONCLUSIONS

The nutritional potential of four *Terminalia* species (*T. arjuna, T. bellirica, T. catappa* and *T. chebula*) was investigated with a view to their valorization as a new source of nutrients. All the species examined, especially *T. arjuna*, showed high concentrations of phenols and macro- and micronutrients. The highest TPC and Fla contents occurred in the seed coats and leaves. Elements i.e. P, S, K, and Rb appeared hyperaccumulated in the four *Terminalia* species. The differences in the FTIR spectra for seed kernels, seed coats and leaves have been referred to the different contents in some components (unsaturated oils, lignin and flavonoids).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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