

Evaluation of Essential and Toxic Metals in Selected Underutilized Fruits of Agasthyamala Biosphere Reserve, Kerala

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ABSTRACT

Fruits are significant constituents of diet providing micronutrients and the knowledge of elemental composition of fruits is essential for the evaluation of consumption and development of new food products. The present study analysed the elemental composition of ten underutilized edible fruits viz., *Aporosa cardiosperma* (Gaertn.) Merr., *Baccaurea courtallensis* (Wight) Muell. Arg., *Elaeocarpus serratus* L., *Flacourtia montana* Graham., *Phoenix pusilla* Gaertn., *Psidium guineense* Sw., *Spondias pinnata* (L. f.) Kurz., *Syzygium cumini* (L.) Skeels, *Syzygium gardneri* Thwaites and *Syzygium zeylanicum* (L.) DC. from the Kerala part of Agasthyamala Biosphere Reserve. Sodium, potassium and calcium were estimated flame photometrically while the remaining metals viz., magnesium, strontium, chromium, manganese, iron, cobalt, copper, zinc, cadmium and lead by inductively coupled plasma- mass spectrometry and the results were recorded as mg 100g⁻¹ fresh weight. All the fruits analysed are characterised by high nutritious metallic elements and very low concentrations of metallic contaminants and sufficient to fulfill the recommended dietary allowance by Indian Council of Medical Research. Hence, they are safe for human consumption and need to be popularised and recommended for commercial exploitation.

Highlights

- Promote the consumption of underutilized fruits naturally rich in micronutrients.
- Improve the economic, social and nutritional status of the rural community.

Keywords: Agasthyamala Biosphere Reserve, Elements, Flame photometry, Inductively coupled plasma-mass spectrometry, Underutilized fruits

The sacred landscape of Agasthyamala located in the south western portion of the Western Ghats, is one of the important Biosphere Reserves in the world where the indigenous culture, religion and spirituality are associated with the biological diversity. It is known for the largest tracts of untouched rainforest in Peninsular India, which provide a diversity of fruit trees where majority of them remains neglected or underutilized. Underutilized or neglected fruit trees were considered to be an important role in mitigating malnutrition and poverty in developing and under developed countries. These

fruit trees may be neglected due to ignorance, lack of knowledge, availability, difficulty in harvesting and storage (Sundriyal and Sundriyal 2003; Gebauer *et al.* 2007; Badola and Aitken 2010).

The mineral content of fruits can vary according to the plant, maturity, soil conditions, climate, and agricultural practices (Pereira *et al.* 2014). More than forty elements have been considered essential to life systems for the survival of both animals and plants. An element is considered essential when reduction of its exposure below a certain limit results consistently in a reduction in



a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in that organism (Armah *et al.* 2001). The beneficial health effects of fruits and their products depends on the amount consumed in a daily diet, type of fruit and the content of biologically active compounds. The quality of fruit products is diminished with increasing concentration of toxic compounds, environmental pollutants and heavy metals, especially lead and cadmium.

Lead and cadmium toxicity is well documented and is recognized as a major environmental health risk throughout the world. Lead affects humans and animals of all ages, but the affects of lead are most serious in young children (Krejpcio *et al.* 2005). The International Agency for Research on Cancer has identified cadmium as a known human carcinogen (Satarug and Moore 2004). Despite the elements play an important role in human health, little is known about the elemental composition of numerous underutilized fruits. In this context the present investigation is an attempt to gain an insight into the elemental composition of some underutilized fruits widely consumed by ethnic communities of Agasthyamala Biosphere Reserve in Kerala.

MATERIALS AND METHODS

Sample collection

Fresh and ripened fruits of selected species viz., *Aporosa cardiosperma* (Gaertn.) Merr., *Baccaurea courtallensis* (Wight) Muell. Arg., *Elaeocarpus serratus* L., *Flacourtia montana* Graham., *Phoenix pusilla* Gaertn., *Psidium guineense* Sw., *Spondias pinnata* (L. f.) Kurz., *Syzygium cumini* (L.) Skeels, *Syzygium gardneri* Thwaites and *Syzygium zeylanicum* (L.) DC. were collected from Kerala part of Agasthyamala Biosphere Reserve during January 2016 to September 2017. Collected fruits were botanically identified with the help of local floras (Sasidharan 2004; Nayar *et al.* 2006).

Sample preparation

The freshly collected samples were washed with distilled water to eliminate soil and other extraneous material and removed the water quickly with a blotting paper. The edible portion was separated

from the fruit, homogenized and accurate amount was weighed as required for different analysis.

Elemental analysis

Elemental composition of the selected underutilized fruits were analysed according to the standard methods of the Association of Official Analytical Chemists (AOAC 2016). Sodium, potassium and calcium were estimated by using flame photometry. For standardization, various concentrations of Na, K and Ca were prepared by ranging 20 ppm, 40 ppm, 60 ppm and 80 ppm by diluting stock solutions of 1000 ppm sodium chloride, potassium chloride and calcium carbonate, respectively. 5 g of fruit sample made into ash at 525°C. Boiled the ash with hydrochloric acid (1:4) solution. Filtered to a volumetric flask and made up to 100 ml with distilled water. From this taken 2 ml and made up to 25 ml with distilled water. Read the concentration by aspirating sample and reference standard solution in a flame photometer. Magnesium, strontium, chromium, manganese, iron, cobalt, copper, zinc, cadmium and lead were estimated by using inductively coupled plasma mass- spectrometry (ICP-MS). For standardization, various concentrations of multi element standard solutions were prepared by ranging 0.5 ppb, 5.0 ppb, 50 ppb, 100 ppb, 200 ppb, 250 ppb by diluting multi element stock solution of 1 ppm using ultra pure water. 0.25 g of fruit sample was taken into the digestion tube. Added 5 ml concentrated nitric acid, 0.5 ml concentrated hydrochloric acid and 1 ml hydrogen peroxide. Mixed well and digested by using micro wave digestion system (MDS). After digestion keep sometime for cooling and made up to 50 ml with ultrapure water. Read the concentration by aspirating sample and reference standard solution in ICP-MS. The results obtained were recorded as mg 100g⁻¹ fresh weight.

Statistical analysis

Statistical analyses were done by SPSS version 16.0 software for windows. One way analysis of variance (ANOVA) along with Duncan's multiple range tests at P < 0.05 was used to determine statistically significant differences in the mean concentrations of elements between the investigated underutilized fruits. Pearson correlation analysis was utilized to determine the inter element association in the

fruits. Principal component analysis (PCA) was used to identify the principal characters which account for the major variation among the fruits and hierarchical cluster analysis (HCA) using Ward's method was applied to identify homogeneous groups in the investigated fruits.

RESULTS AND DISCUSSION

Quantification of selected metals

Mean concentration of selected elements in the investigated fruits are summarized in Table 1. Potassium and sodium are the most abundant elements present in the studied fruits, which were found 110.00 ± 0.39 mg $100g^{-1}$ to 590.00 ± 0.07 mg $100g^{-1}$ and 10.00 ± 0.02 mg $100g^{-1}$ to 680.00 ± 1.07 mg $100g^{-1}$ respectively. The Na/K ratio in our body is very important to control high blood pressure and the ratio should be less than one (Akubugwo *et al.* 2007). In the present study, *Aporosa cardiosperma* (Gaertn.) Merr., *Baccaurea courtallensis* (Wight) Muell. Arg., *Phoenix pusilla* Gaertn., *Spondias pinnata* (L. f.) Kurz., *Syzygium cumini* (L.) Skeels, *Syzygium gardneri* Thwaites had the Na/K ratio less than one that indicates the consumption of these fruits might be able to control the high blood pressure

of our body. According to Indian Council of Medical Research (ICMR) the recommended dietary allowance (RDA) of sodium for male and female is 2092 mg day^{-1} and 1902 mg day^{-1} , respectively. RDA for potassium is 3750 mg day^{-1} for male and 3225 mg day^{-1} for female (ICMR, 2010). The concentration of calcium was found highest in *Elaeocarpus serratus* L. (360.00 ± 0.47 mg $100g^{-1}$) and lowest in *Baccaurea courtallensis* (Wight) Muell. Arg. (180.00 ± 0.41 mg $100g^{-1}$). Calcium is an essential mineral for bone formation, the deficiency of which leads to reduced bone formation, osteoporosis and proneness to bone fracture (Narasinga Rao 2010). The daily calcium intake recommended by ICMR for both male and female is 600 mg day^{-1} . The magnesium concentration analyzed in this study was highest in *Elaeocarpus serratus* L. (45.58 ± 0.57 mg $100g^{-1}$) and lowest in *Syzygium zeylanicum* (L.) DC. (17.40 ± 0.01 mg $100g^{-1}$). Magnesium is an essential cation playing a crucial role in many physiological functions. It is critical in energy requiring metabolic processes, in protein synthesis, membrane integrity, nervous tissue conduction, neuromuscular excitability, muscle contraction, hormone secretion, and in intermediary metabolism (Laires *et al.* 2004). According to ICMR, RDA of magnesium for male

Table 1: Elemental composition of selected underutilized fruits (mg $100g^{-1}$ fresh weight)

Fruit species	Na	K	Mg	Ca	Sr	Cr	Mn	Fe	Co	Cu	Zn	Cd	Pb
<i>A. cardiosperma</i>	40.00 ± 0.07^c	160.00 ± 0.12^b	44.93 ± 0.03^h	230.00 ± 0.08^c	0.14 ± 0.01^d	0.05 ± 0.00^{bc}	0.66 ± 0.01^d	0.48 ± 0.01^{abc}	N. D.	0.12 ± 0.06^b	0.10 ± 0.01^{bc}	N. D.	0.01 ± 0.00
<i>B. courtallensis</i>	20.00 ± 0.34^b	110.00 ± 0.39^a	21.21 ± 0.73^d	180.00 ± 0.41^a	0.11 ± 0.01^c	0.03 ± 0.00^a	0.11 ± 0.02^a	0.38 ± 0.02^{ab}	N. D.	0.13 ± 0.02^{bc}	0.10 ± 0.01^{bc}	N. D.	0.01 ± 0.00
<i>E. serratus</i>	680.00 ± 1.07^h	400.00 ± 1.69^g	45.58 ± 0.57^h	360.00 ± 0.47^i	0.10 ± 0.02^c	0.12 ± 0.01^f	1.95 ± 0.03^j	13.21 ± 0.34^h	N. D.	0.13 ± 0.02^{bc}	0.15 ± 0.02^d	N. D.	0.01 ± 0.00
<i>F. montana</i>	640.00 ± 0.47^g	320.00 ± 0.39^d	18.52 ± 0.56^b	260.00 ± 0.25^e	0.34 ± 0.02^f	0.12 ± 0.01^f	0.72 ± 0.06^e	0.61 ± 0.01^c	0.01 ± 0.00	0.23 ± 0.01^e	0.37 ± 0.01^g	N. D.	0.01 ± 0.00
<i>P. pusilla</i>	10.00 ± 0.05^a	380.00 ± 0.02^f	22.40 ± 0.02^e	340.00 ± 0.02^h	0.05 ± 0.01^a	0.09 ± 0.01^d	0.62 ± 0.02^c	1.23 ± 0.03^d	N. D.	0.15 ± 0.01^c	0.13 ± 0.01^{cd}	N. D.	0.01 ± 0.00
<i>P. guineense</i>	500.00 ± 0.12^e	420.00 ± 0.61^h	26.21 ± 0.32^f	290.00 ± 0.57^f	0.07 ± 0.01^{ab}	0.06 ± 0.01^c	0.49 ± 0.02^b	0.35 ± 0.01^a	N. D.	0.21 ± 0.02^d	0.29 ± 0.03^f	N. D.	N. D.
<i>S. pinnata</i>	10.00 ± 0.02^a	330.00 ± 0.04^e	20.15 ± 0.03^c	300.00 ± 0.02^g	0.13 ± 0.01^d	0.14 ± 0.01^g	0.91 ± 0.01^f	5.01 ± 0.03^g	N. D.	0.29 ± 0.01^f	0.21 ± 0.04^e	N. D.	0.01 ± 0.00
<i>S. cumini</i>	340.00 ± 0.44^d	590.00 ± 0.07^i	22.41 ± 0.49^e	250.00 ± 0.56^d	0.08 ± 0.01^b	0.11 ± 0.01^e	1.43 ± 0.02^i	2.42 ± 0.01^f	N. D.	0.15 ± 0.01^c	0.21 ± 0.01^e	N. D.	0.01 ± 0.00
<i>S. gardneri</i>	500.00 ± 0.16^e	510.00 ± 0.43^i	30.51 ± 0.66^g	220.00 ± 0.31^b	0.18 ± 0.01^e	0.04 ± 0.01^b	1.35 ± 0.03^h	0.56 ± 0.01^{bc}	N. D.	0.08 ± 0.01^a	0.05 ± 0.01^a	N. D.	N. D.
<i>S. zeylanicum</i>	630.00 ± 0.06^f	310.00 ± 0.04^c	17.40 ± 0.01^a	250.00 ± 0.12^d	0.06 ± 0.01^{ab}	0.09 ± 0.01^d	1.10 ± 0.01^g	1.63 ± 0.01^e	N. D.	0.12 ± 0.01^b	0.10 ± 0.01^b	N. D.	0.01 ± 0.00

a. Values are means ($n = 3$) \pm SD b. Values with different superscript are significantly different at $p < 0.05$ c. N.D= Not detected



and female is 340 mg day^{-1} and 310 mg day^{-1} , respectively.

The iron content in the fruits were ranged from $0.35 \pm 0.01 \text{ mg } 100\text{g}^{-1}$ to $13.21 \pm 0.34 \text{ mg } 100\text{g}^{-1}$. Iron is important in the diet for the formation of haemoglobin, normal functioning of the central nervous system and in the metabolism of carbohydrate, protein and fat (Gupta, 2014). According to ICMR, RDA of iron is 17 mg day^{-1} for male and 21 mg day^{-1} for female. Manganese was found highest in *Elaeocarpus serratus* L. ($1.95 \pm 0.03 \text{ mg } 100\text{g}^{-1}$) and lowest in *Baccaurea courtallensis* (Wight) Muell. Arg. ($0.11 \pm 0.02 \text{ mg } 100\text{g}^{-1}$). Manganese is an element of vital importance, contributing to structural components and the activation of several enzymes (Altundag and Tuzen 2011). According to ICMR, RDA of manganese for an adult to be between $2\text{-}5 \text{ mg day}^{-1}$. Zinc is an essential mineral that plays catalytic, structural and regulatory roles as an integral part of many enzymes in human body. It is essential for normal growth, mental ability, immune system, reproduction and healthy function of the heart (Deshpande *et al.* 2013). Nevertheless, higher concentrations of zinc can be toxic to the organism (Rajkovic *et al.* 2008). The concentration of zinc was observed between $0.05 \pm 0.01 \text{ mg } 100\text{g}^{-1}$ and $0.37 \pm 0.01 \text{ mg } 100\text{g}^{-1}$. According to ICMR, RDA of zinc for male and female is 12 mg day^{-1} and 10 mg day^{-1} , respectively. Copper is an important trace mineral for health, assisting in the formation of haemoglobin and takes part in many different enzyme activities (Osredkar *et al.* 2011). In some cases it may be toxic when its concentration exceeds the safe limit (Ogwok *et al.* 2014). The concentration of copper was ranged from $0.08 \pm 0.01 \text{ mg } 100\text{g}^{-1}$ to $0.29 \pm 0.01 \text{ mg } 100\text{g}^{-1}$. According to ICMR recommended dietary allowance of copper is 1.35 mg day^{-1} .

Cobalt is a necessary cofactor for making the thyroid hormone thyroxin. Cobalt has also been used in anaemia treatment as it causes the red blood cells production. The toxicity of cobalt is quite low compared to that of many other metals (Song *et al.* 2003). Strontium can improve the cellular makeup of bones and teeth by preventing tooth decay or soft bones. It has not been proven that low strontium can cause the negative outcomes similar to calcium (Stojanovic *et al.* 2014). In the present study cobalt ($0.01 \text{ mg } 100\text{g}^{-1}$) was found only in *Flacourtia*

montana Graham and the strontium concentration was ranged from $0.05 \pm 0.01 \text{ mg } 100\text{g}^{-1}$ to $0.34 \pm 0.02 \text{ mg } 100\text{g}^{-1}$. A safe recommended dietary allowance for cobalt and strontium hasn't been set yet. Chromium is considered as a biological and pollution significant element (Jayana *et al.* 2009). Chromium, in the trivalent form (Cr(III)), is an important component of a balanced human and animal diet and its deficiency causes disturbance to the glucose and lipids metabolism in humans and animals. In contrast, hexavalent chromium (Cr(VI)) is highly toxic carcinogen and may cause death to animals and humans if ingested in large doses. (Zayed and Terry 2003). The concentration of chromium was ranged from $0.03 \pm 0.00 \text{ mg } 100\text{g}^{-1}$ to $0.14 \pm 0.01 \text{ mg } 100\text{g}^{-1}$. According to ICMR, RDA of chromium is 0.03 mg day^{-1} . The chromium content was exceeded the permissible limit in all fruits except *Baccaurea courtallensis* (Wight) Muell. Arg. These high amounts of chromium might be accumulated in the fruits due to the contamination of soil, wastewater or industrial effluents (Rehman *et al.* 2008; Ramesh *et al.* 2012). Lead and cadmium are very harmful elements for human body especially in high concentration (Hamurcu 2010). None of the fruits contained cadmium but lead ($0.01 \text{ mg } 100\text{g}^{-1}$) was detected in almost all selected fruits except *Psidium guineense* Sw. and *Syzygium gardneri* Thwaites, within the FSSAI permissible limit $0.01 \text{ mg } 100\text{g}^{-1}$ in fruits (FSSAI 2011).

Inter element correlation

Inter element associations in the investigated fruits is presented in Table 2. High positive correlation was observed between Co and Sr ($r = 0.877, p < 0.01$), Zn and Cu ($r = 0.755, p < 0.01$), Fe and Mn ($r = 0.722, p < 0.01$), Zn and Co ($r = 0.694, p < 0.01$), Fe and Ca ($r = 0.663, p < 0.01$), Ca and Cr ($r = 0.661, p < 0.01$), Mn and K ($r = 0.640, p < 0.01$) and Cu and Cr ($r = 0.622, p < 0.01$). Moderate positive correlation was observed between Mn and Na ($r = 0.563, p < 0.01$), Fe and Cr ($r = 0.563, p < 0.01$), Fe and Mg ($r = 0.492, p < 0.01$), Pb and Cr ($r = 0.477, p < 0.01$), Mn and Cr ($r = 0.465, p < 0.01$), Zn and Cr ($r = 0.463, p < 0.01$), Zn and Sr ($r = 0.461, p < 0.05$), Na and K ($r = 0.436, p < 0.05$), Mn and Ca ($r = 0.426, p < 0.05$), Mn and Mg ($r = 0.405, p < 0.05$), Cu and Co ($r = 0.394, p < 0.05$) and Na and Co ($r = 0.369, p < 0.05$) while negative correlation was observed between Pb and

Table 2: Inter element association in the selected underutilized fruits

Element	Na	K	Mg	Ca	Sr	Cr	Mn	Fe	Co	Cu	Zn	Pb
Na	1.000											
K	0.436*	1.000										
Mg	0.062	-0.077	1.000									
Ca	0.173	0.348	0.219	1.000								
Sr	0.246	-0.143	-0.078	-0.254	1.000							
Cr	0.169	0.326	-0.168	0.661**	0.134	1.000						
Mn	0.563**	0.640**	0.405*	0.426*	-0.086	0.465**	1.000					
Fe	0.293	0.182	0.492**	0.663**	-0.170	0.563**	0.722**	1.000				
Co	0.369*	-0.080	-0.285	-0.051	0.877**	0.294	-0.144	-0.174	1.000			
Cu	-0.160	-0.054	-0.387*	0.328	0.283	0.622**	-0.264	0.060	0.394*	1.000		
Zn	0.276	0.154	-0.319	0.244	0.461*	0.463**	-0.164	-0.040	0.694**	0.755**	1.000	
Pb	-0.297	-0.408*	-0.073	0.124	0.027	0.477**	0.013	0.281	0.167	0.162	0.007	1.000

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level

Table 3: Principal components in the selected underutilized fruits

Parameters	Component			
	PC1	PC2	PC3	PC4
Sodium	0.250	0.496	-0.225	0.662
Potassium	0.339	-0.118	0.122	0.841
Magnesium	0.271	-0.036	-0.757	-0.063
Calcium	0.833	-0.155	0.166	0.115
Strontium	-0.127	0.929	0.063	-0.064
Chromium	0.850	0.195	0.403	-0.048
Manganese	0.694	0.027	-0.456	0.462
Iron	0.881	-0.039	-0.334	-0.019
Cobalt	-0.007	0.931	0.294	-0.035
Copper	0.313	0.231	0.812	-0.203
Zinc	0.205	0.561	0.674	0.126
Lead	0.404	0.100	0.023	-0.757
Eigenvalue	3.474	3.221	1.948	1.401
% of Variance	28.950	26.844	16.232	11.672
Cumulative %	28.950	55.794	72.026	83.698

K ($r = -0.408$, $p < 0.05$) and Cu and Mg ($r = -0.387$, $p < 0.05$). The high correlation among the macro and essential trace elements indicate that these fruits are the sources of multiple elements. Consumers of these underutilized fruits will be obtaining multiple macro and essential trace elements in their diet that will improve their overall health.

Principal component analysis

PCA was performed by varimax normalized rotation and the results including the loading, eigenvalue and variance contribution rate summarized in Table 3. Scree plot in Fig. 1 reveals the first four PCs with

eigenvalues more than one are the most significant components and Fig. 2 represents the component plot in rotated space. Four PCs were obtained with eigenvalue greater than one with total variance of 83.70%. These are considered responsible for the variation among the fruits. PC1 explains 28.95% of the total variance, and has high positive loading on iron (0.881), chromium (0.850), calcium (0.833) and manganese (0.694). PC2 has high positive loading on cobalt (0.931) and strontium (0.929), which explains 26.84% of total variance. PC3 has positive loading on copper (0.812) and zinc (0.674) while a negative loading on magnesium (-0.757), and explains 16.23%

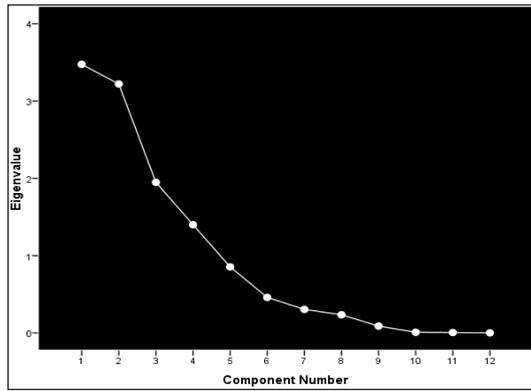


Fig. 1: PCA- Scree plot

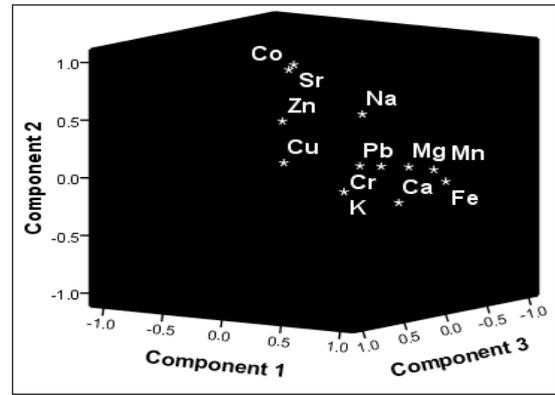


Fig. 2: PCA- Component plot in rotated space

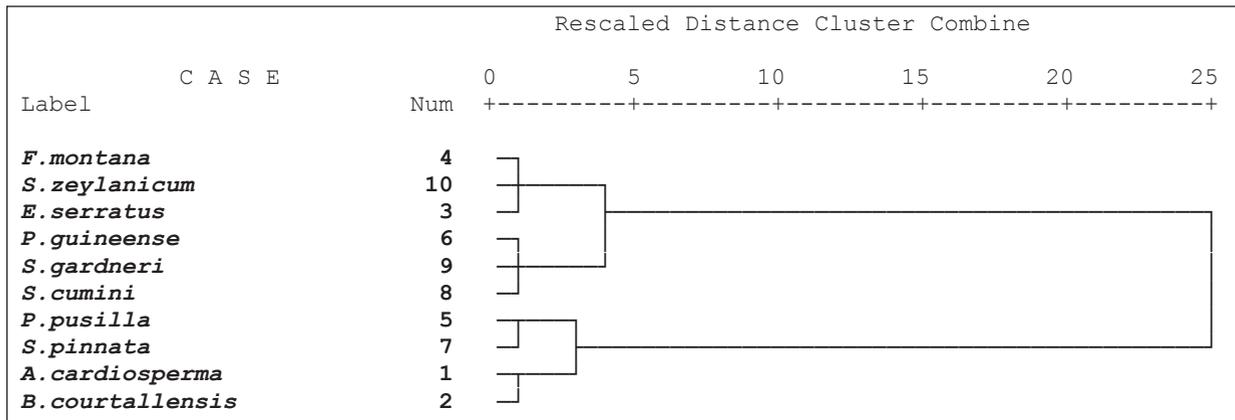


Fig. 3: Dendrogram of selected underutilized fruits based on elemental composition

of the total variance. PC4 explains 11.67% of the total variance and has positive loading on potassium (0.841) and sodium (0.662) while a negative loading on lead (-0.757).

Hierarchical cluster analysis

The ten underutilized fruits on the basis of resemblance in their elemental composition are grouped into three distinct clusters as illustrated in Fig. 3. Cluster I comprised the fruits, *Aporosa cardiosperma* (Gaertn.) Merr, *Baccaurea courtallensis* (Wight) Muell. Arg., *Phoenix pusilla* Gaertn. and *Spondias pinnata* (L. f.) Kurz. while cluster II consist of *Elaeocarpus serratus* L., *Flacourtia montana* Graham. and *Syzygium zeylanicum* (L.) DC. and cluster III contained *Psidium guineense* Sw., *Syzygium cumini* (L.) Skeels and *Syzygium gardneri* Thwaites.

The results indicate that the investigated fruits contain appreciable quantities of essential metals particularly, Na, K, Ca, Mg, Fe, Mn, Cu, Zn and low concentrations of toxic metal Pb, within the permissible limit of FSSAI and hence they are safe

for human consumption. These underutilized fruits are very good source of multiple elements and sufficient to fulfill the daily intake of micronutrients recommended by ICMR and regular consumption of these fruits may be helpful in overcoming the micronutrient deficiencies among the rural poor.

CONCLUSION

The underutilized fruits consumed by ethnic communities of Agasthyamala Biosphere Reserve are enriched with elements of vital importance in human metabolism. Elemental composition of fruits is most essential for developing new strategies of drug design based on natural resources and the data obtained in the present study will be useful in the synthesis of new herbal medicines and functional foods with various combinations of underutilized fruits to prevent and cure micronutrient deficiency syndrome. Promotion of underutilized fruit processing in its proper perspective will open up new horizons in the socioeconomic development of the rural community and that will enhance food



security, employment and income of the people of the country.

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