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Analysis of macro and micro elemental composition of different extracts and finished products of the medicinal Herb – *Terminalia bellirica*

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Abstract: *Terminalina bellirica* is considered to be a sacred plant with five distinctive tastes (sweet, sour, salty, bitter, and pungent). It stimulates the proper functioning of the digestive system, sensory organs and it is very effective in treating several medical conditions. Four types of extracts were prepared (aqueous extracts of *T. bellirica* fruit, alcoholic extract of *T. bellirica* fruit, alcoholic extract of seed oil from *T. bellirica* nuts, and alcoholic extract of *T. bellirica* seed kernel). The elemental composition of *T. bellirica* was determined by ICP-OES. Macro elements like Ca (calcium), Na (sodium), and K (potassium) were found to be abundant in the studies samples. The quantity of Ca, Na, and K in the aqueous extracts of *T. bellirica* fruit, finished products of *T. bellirica*, alcoholic extract of *T. bellirica* fruit, alcoholic extract of seed oil from *T. bellirica* nuts, and alcoholic extract of *T. bellirica* seed kernel was found to be (723.51-787.39, 347.80-478.90, 50.72, 120, and 300 mg/kg), (603.45-636.79, 241.76-370.00, 9.58, 180, and 200 mg/kg), and (151.23-165.73, 132.70-154.40, 4.26, 200, and 1,150 mg/kg), respectively. An Na/K ratio < 1.0 could prevent high blood pressure. The Na/K ratio in the alcoholic extract of *T. bellirica* fruit was found to be 0.51. Similarly, a Ca/P ratio > 1.0 is biologically relevant as a Ca/P ratio < 0.5 correlates with several health problems. The Ca/P ratio in the alcoholic extract of *T. bellirica* fruit was found to be 6.68. Hence, the measured Na/K and Ca/P ratios are in agreement with the recommended ratios. Our study supports the claims that *T. bellirica* extracts and finished products are of considerable therapeutic value for the prevention and/or treatment of various medical conditions. Moreover, the findings of our study will be helpful to ensure the quality and verify purification of natural products in different ayurvedic formulations.

Keywords: *T. bellirica*, microwave digestion, ICP-OES, trace elements, Na/K ratio.

1. Introduction

Bellirica Myrobalan is commonly known as Bibitaki in Sanskrit and Bahera by local name which is an edible plant distributed throughout the central Asia [1]. It has been used as a folk medicine for more than thousands of years in Asia due to its tonic effects [2]. Bibitaki is a traditional name given to *T. bellirica*, which is one of the best single herbs controlling Kapha and it is well reputed as rasayana in Ayurvedic medicine. In Hindu language, Bibitaki means "fearless", and *T. bellirica* is considered a sacred herb among Hindu religious people of North India. It was introduced in Europe as an herbal medicine by Arabs. Recent research studies identified interesting phytochemicals present in *T. bellirica*, which may explain the reported biological activities of this plant's extracts and their use in traditional medicine [3]. Similarly, recent reports also highlighted the medicinal potential of certain Terminalia species cultivated in Africa, Australia, and America [4]. It is known to possess medicinal activities such as analgesic, antioxidant, hepatoprotective, anti-bacterial, anti-cancer, and immunomodulatory activities [5].

Accumulation of stones or calculi in the urinary, digestive, and respiratory tracts could be removed or expelled by the use of *T. bellirica* aqueous extract [6]. It is considered to be the strong rejuvenator to the human body and takes care of voice, vision and hair growth [7]. Because of its dual behavior like laxative and astringent and hence it purges the bowels and also toning the tissues of the digestive tract simultaneously.

It is one of the constituents of the famous preparation "Triphala", which finds use in a wide array of therapeutic areas ranging from hair care, laxative, headache, leucorrhoea, liver diseases to gastro-

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intestinal complaints [8,9]. Its fruit has been used in traditional medicine for anemia, asthma, cancer, colic, constipation, diarrhea, dysuria, headache, hypertension, inflammations, and rheumatism [10,11]. The leaves and fruits of *T. bellirica* possess antioxidant activity [12,13]. The aqueous and alcoholic extracts of the individual constituents of *T. bellirica* were shown to improve macrophage functions with regard to free radical scavenging and reactive oxygen species neutralization [10,14]. Surprising results were obtained when using ophthalmic drops (herbal eye drops) containing *T. bellirica* (Gaertn.) fruit-bearing part, displaying myocardial depressive activity especially in the case of myopia, pterygium, corneal opacity, unripe cataract, as well as other acute and chronic conditions [15]. Hypercholesterolemic animals showed marked reduction in their lipid levels when administered with *T. bellirica* [16]. Most recent research studies revealed that *T. bellirica* has the ability to lower the lipid levels in the liver and heart of the human body [17]. The risk associates with heart and liver is well protected due its strong ability in preventing heart and liver fat congestions.

Glucoside, tannins, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulanic acid are the main active phytoconstituents of medicinal importance. These phytoconstituents are responsible for many of the reported pharmacological roles [18,19]. Other studies revealed that extracts of *T. bellirica* leaves are of important therapeutic value as they exhibit antioxidant and anti-fungal activities [20, 21].

The therapeutic nature of *T. bellirica* could also be looked in terms of some essential elements present in it. Macro elements like Ca, Mg, Na, and K are present in abundant quantities and are very unique when compared to other Terminalia species. In this study, the aqueous and alcoholic extracts of *T. bellirica* fruit and alcoholic extracts of *T. bellirica* seed oil and seed kernel were subjected to elemental determination by Inductively Coupled Plasma instrument and the elemental profile was created and calculated the concentration of each element and mean values were determined and tabulated and also regression analysis was performed for the data collected for each element.

2. Material and Methods

2.1. Collection of Samples

Well-matured, healthy, and dried fruits of *T. bellirica* were collected from different locations in India (April to June) and its mineral contents were quantified using ICP-OES.

Source I: Tambaram, India (12° 55' 22.4940" N and 80° 7' 38.8452" E)

Source II: Bangalore, India (12.9716° N and 77.5946° E)

Source III: Coimbatore, India (11° 0' 16.4016" N and 76° 57' 41.8752" E)

Source IV: Nagpur, India (21° 8' 47.8788" N and 79° 5' 19.8960" E)

The standard aqueous extracts of *T. bellirica* (TBEs) are shown in Table 1. The finished products consisting of *T. bellirica* as a major ingredient (TBFs) are shown in Table 2.

Table 1. Standard aqueous extracts of *T. bellirica* (TBEs).

Code	Source	Mass (g)	Volume (ml)
TBE-1	Tambaram	1.2384	50.0
TBE-2	Bangalore	1.2276	50.0
TBE-3	Coimbatore	1.2143	50.0
TBE-4	Nagpur	1.2198	50.0

Table 2. Finished products consisting of *T. bellirica* as a major ingredient (TBFs).

Code	Source	Mass (g)	Volume (ml)
TBF-1	Chennai	1.2303	50.0
TBF-2	Coimbatore	1.2163	50.0
TBF-3	Bangalore	1.2414	50.0

2.2. Solutions and Reagents

Standard solutions were prepared by diluting individual metal standard solution (1,000 µg/ml) procured from BDH Middle East L.L.C. with 1.0 M HNO_3. The four types of extracts (aqueous extracts of *T. bellirica* fruit, alcoholic extract of *T. bellirica* fruit, alcoholic extract of seed oil from *T. bellirica* nuts, and alcoholic extract of *T. bellirica* seed kernel) were incinerated in a muffle furnace and made into ash, which were then digested with HNO_3 + 30% H_2O_2 and prepared the solutions.

2.3. Preparation of Sample by Microwave Digestion Method

The samples were prepared by the microwave digestion method as previously described [22]. Briefly,

all extracts were weighed accurately and transferred into seven fluorocarbon microwave vessels. Next, 10 ml concentrated HNO_3 was added to each vessel. The vessels were sealed and properly placed in the microwave system (Anton Paar microwave digester). A blank sample was also prepared simultaneously by placing 10 ml of concentrated HNO_3 in one of the empty fluorocarbon microwave vessel and placed it in the microwave system after sealing it tightly. All the samples and blank were digested at 175°C for 10 minutes. After cooling it to a sufficient time, carefully uncapped and vented each vessel in fume cupboard. Contents of each vessel then transferred to 50 ml volumetric flask and made up to the mark with Mille-Q water.

2.4. Preparation of Reference Standard Solution

Standard solutions of each element were separately prepared using reference standard metal solutions. The procedure followed was as per the Analysis Guidebook of Shimadzu Solutions for Science. The solutions prepared were placed in tightly capped plastic bottles and used within a day of their preparation.

2.5. Preparation of Reagent Blank Solution

10 ml of conc. nitric acid and 2 ml of 30% H_2O_2 were quantitatively added into 50 ml volumetric flaks and diluted with de-ionized water to the mark. These solutions were served as blank solution.

2.6. Determination of Elements Concentration

Elemental profile of *T. bellirica* was determined quantitatively by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) (VARIAN INC. - liberty series, axial viewed plasma, sequential type ICP-OES spectrometer, wavelength range 189-940 nm). Using reference standards, the calibration curves were obtained for all the elements. The sample solutions were adequately diluted to keep the absorbance in the linear range of measurement.

2.7. Statistical Analysis

All assays were carried out in triplicates and values were obtained by calculating the average of five experiments. Data are presented as mean ± SEM.

The SEM is calculated by dividing the SD (σ) by the square root of n (number of samples).

The formula to find the sample mean

$$\mu_x = \frac{\sum_1^n x_i}{n}$$

Formula to estimate sample standard deviation

$$s = \sqrt{\frac{\sum_1^n (x_i - \mu_x)^2}{n-1}}$$

Formula to estimate standard error (SE) of mean

$$sE_{\mu_x} = \frac{s}{\sqrt{n}}$$

3. Results and Discussion

An appropriate preparation method was chosen, a simultaneous quantitative mineralization and dissolution made by using relevant chemical reagents and physical means. Partial dissolution could occur due to high contents of silicates and that may lead to poor recoveries of many elements, especially those that are relatively abundant in plant media and partly associated with silicate. Besides aluminum and other elements such as Fe and B also results in poor recoveries in case of incomplete dissolution of sample. After the preparation procedure applied in this work, the sample matrix was largely simplified and the resulting solution was found to be clear, colorless, and odorless with no observed residues and ensuring complete dissolution was achieved in most cases.

With regard to ICP analysis, no specific precautions have to be taken as long as the measured concentrations satisfy specific criteria like sensitivity, detection limits, and working range. Moreover, avoiding interfering substances is of great importance. With regard to ICP-OES analysis, the procedure should be performed under the optimal plasma conditions. The detection limits of ICP-OES are of mg/l depending on the element of choice and thus enable quantification. The above method of analysis should be validated with the methods to CRM (NIST 1573 - tomato leaves) from mineralization, dissolution, and measurements. ICP-OES analysis revealed that the found values were very close to the certified values of the examined elements (Table 3)

Table 3. Concentration values of elements of CRM (NIST 1573 - tomato leaves) in mg/kg.

Element	Found Value (mg/kg)	Certified Value (mg/kg)	Recovery (%)
Cu	7.55	4.70 ± 0.14	160.6
Zn	36.43	30.9 ± 0.70	117.9
Mn	225.8	246 ± 8.00	91.8
Fe	304.0	368 ± 7.00	82.6
Co	0.64	0.57 ± 0.04	112.3

Cr	2.23	1.99 ± 0.06	112.2
Ni	0.90	1.59 ± 0.04	56.60
Pb	1.04	Not available	Not available
Ca	5000	5005 ± 90	101.0
Al	526.4	598 ± 2.00	88.03
Cd	1.33	1.52 ± 0.03	87.5
K	2460	2700 ± 50	91.1
Na	4000	4136 ± 400	294.5

Simultaneously, an external calibration was also carried out and statistical parameters like regression and correlation analysis were performed and found the correlation coefficient(r) value in the range of 0.9981-0.9999. The results of elemental analysis of the aqueous extracts of *T. bellirica* fruit (TBE-1, TBE-2, TBE-3, and TBE-4) and the finished products of *T. bellirica* (TBF-1, TBF-2, and TBF-3) are shown in Table 4 and Table 5, respectively.

Table 4. Elemental composition of the aqueous extracts of *T. bellirica* fruit (TBE-1, TBE-2, TBE-3, and TBE-4).

Element	TBE-1 (mg/kg)	TBE-2 (mg/kg)	TBE-3 (mg/kg)	TBE-4 (mg/kg)
Ca	723.51 ± 0.010	762.54 ± 0.010	787.39 ± 0.008	742.74 ± 0.005
Mg	415.63 ± 0.097	467.56 ± 0.003	433.49 ± 0.013	424.24 ± 0.003
Na	636.79 ± 0.000	615.43 ± 0.003	624.36 ± 0.009	603.45 ± 0.014
K	156.86 ± 0.024	165.73 ± 0.017	151.23 ± 0.012	154.45 ± 0.008
Al	43.1650 ± 0.003	39.3160 ± 0.026	47.3870 ± 0.037	53.3760 ± 0.045
B	3.6556 ± 0.0010	2.9445 ± 0.0008	4.1345 ± 0.0014	2.7600 ± 0.0005
Ba	1.0241 ± 0.0008	1.0823 ± 0.0014	1.0837 ± 0.0015	1.0923 ± 0.0018
Cd	0.0600 ± 0.0005	0.0400 ± 0.0003	0.0500 ± 0.0004	0.0600 ± 0.0005
Co	0.0137 ± 0.0001	0.0277 ± 0.0003	0.0349 ± 0.0005	0.0146 ± 0.0002
Cr	0.0489 ± 0.0008	0.0527 ± 0.0011	0.0515 ± 0.0010	0.0493 ± 0.0010
Cu	1.1494 ± 0.003	1.1087 ± 0.025	1.1097 ± 0.027	1.1503 ± 0.022
Fe	3.1559 ± 0.041	3.2974 ± 0.062	3.2815 ± 0.048	3.3074 ± 0.075
Li	0.4829 ± 0.0028	0.4935 ± 0.0029	0.4318 ± 0.0025	0.4719 ± 0.0028
Mn	2.7114 ± 0.000	2.7700 ± 0.000	2.9200 ± 0.005	2.8600 ± 0.003
Mo	0.0119 ± 0.0012	0.0213 ± 0.0022	0.0135 ± 0.0013	0.0157 ± 0.0018
Ni	0.0802 ± 0.0008	0.0754 ± 0.0007	0.0954 ± 0.0010	0.0698 ± 0.0005
P	0.2946 ± 0.0015	0.3457 ± 0.0019	0.2765 ± 0.0012	0.3249 ± 0.0017
Pb	0.6347 ± 0.0010	0.6328 ± 0.0008	0.5327 ± 0.0006	0.5328 ± 0.0006
Rb	152.86 ± 0.0085	160.86 ± 0.0089	152.86 ± 0.0085	155.65 ± 0.0087
S	0.0267 ± 0.0004	0.0261 ± 0.0004	0.0235 ± 0.0003	0.0219 ± 0.0001
Se	0.3961 ± 0.0013	0.3912 ± 0.0011	0.4019 ± 0.0015	0.4170 ± 0.0017
Si	7.6356 ± 0.0036	7.7570 ± 0.0038	7.9767 ± 0.0047	7.4246 ± 0.0032
Sn	0.1811 ± 0.0008	0.1834 ± 0.0010	0.1891 ± 0.0012	0.1795 ± 0.0006
Sr	8.6401 ± 0.0024	8.4790 ± 0.0021	8.4387 ± 0.0016	8.4437 ± 0.0018
Te	0.3223 ± 0.0005	0.4308 ± 0.0014	0.3483 ± 0.0009	0.3454 ± 0.0008
Ti	7.1562 ± 0.0023	7.1593 ± 0.0025	7.5640 ± 0.0038	7.5757 ± 0.0041
V	0.0115 ± 0.0002	0.0124 ± 0.0003	0.0138 ± 0.0005	0.0153 ± 0.0009
Zn	1.3426 ± 0.0013	1.4532 ± 0.0021	1.3679 ± 0.0018	1.9345 ± 0.0037

An examination of the data from Table 4 reveals that this medicinal plant containing elements like K, Mg, Mn, Na, B, Ba, Fe, Zn, Cu, Cl, Al, Cr, Ca, Cd, Ni, P, Pb, S, Se, Sn, Sr, Si, Te, Ti, and V in different proportions. All the chosen elements were analyzed by ICP-OES instrument by measuring the absorbance of the species at its resonance wavelength. The variation in elemental concentration is mainly

attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants were cultivated [23]. Other factors responsible for a variation in elemental concentration are preferential absorbability of the plant, use of fertilizers, irrigation water and climatic conditions [24]. The active constituents of the medicinal plants are the metabolic products of the plant cells. A number of trace elements play important roles in metabolism, and they are essential for successful catalysis.

Table 5. Elemental composition of the finished products of *T. bellirica* (TBF-1, TBF-2, and TBF-3).

Element	TBF-1 (mg/kg)	TBF-2 (mg/kg)	TBF-3 (mg/kg)
Ca	347.80 ± 0.142	478.90 ± 0.217	386.50 ± 0.194
Mg	155.40 ± 0.114	194.50 ± 0.165	163.80 ± 0.123
Na	290.45 ± 0.259	370.00 ± 0.328	241.76 ± 0.209
K	154.40 ± 0.172	132.70 ± 0.131	142.45 ± 0.156
Al	15.456 ± 0.055	12.657 ± 0.028	13.654 ± 0.037
B	1.2956 ± 0.0022	1.3856 ± 0.0033	1.2567 ± 0.0019
Ba	0.5640 ± 0.0046	0.6543 ± 0.0057	0.5876 ± 0.0050
Cd	0.0100 ± 0.0001	0.0158 ± 0.0003	0.0186 ± 0.0006
Co	0.0165 ± 0.0010	0.0197 ± 0.0017	0.0178 ± 0.0013
Cr	0.0105 ± 0.0023	0.0130 ± 0.0037	0.0126 ± 0.0031
Cu	0.3954 ± 0.0135	0.4254 ± 0.0147	0.3354 ± 0.0113
Fe	1.4265 ± 0.0275	1.4676 ± 0.0291	1.3778 ± 0.0315
Li	0.1400 ± 0.0016	0.1700 ± 0.0028	0.1100 ± 0.0012
Mn	0.9873 ± 0.0120	0.8765 ± 0.0082	0.9543 ± 0.0107
Mo	0.0976 ± 0.0073	0.0877 ± 0.0061	0.0765 ± 0.0054
Ni	0.0770 ± 0.0015	0.0840 ± 0.0024	0.0710 ± 0.0013
P	0.1974 ± 0.0011	0.2015 ± 0.0009	0.1843 ± 0.0012
Pb	0.2456 ± 0.0135	0.3876 ± 0.0146	0.4567 ± 0.0158
Se	0.1543 ± 0.0105	0.1238 ± 0.0073	0.1287 ± 0.0176
Si	0.4270 ± 0.0143	0.3970 ± 0.0131	0.4730 ± 0.0152
Sn	0.0180 ± 0.0012	0.0220 ± 0.0017	0.0230 ± 0.0019
Sr	1.2754 ± 0.0172	1.3546 ± 0.0215	1.2137 ± 0.0156
Te	0.0190 ± 0.0016	0.0120 ± 0.0011	0.0260 ± 0.0027
Ti	1.5300 ± 0.0341	1.6700 ± 0.0475	1.4100 ± 0.0292
V	0.0086 ± 0.0009	0.0092 ± 0.0010	0.0100 ± 0.0012
Zn	4.4000 ± 0.0572	3.0000 ± 0.0433	4.9000 ± 0.0631

Trace elements or micronutrients are essential to human life and they are supplemented in the form of minerals and vitamins. These can work as co-factors of enzyme synthesis and plays a vital role in organizing the cell structure and its membrane. Trace element are toxic when its optimum levels exceeds and lead to metabolic disorders when it is deficient [25]. In total, these trace elements are most important for human metabolism. These elements are crucial for normal biological processes such as metabolism, growth, and development.

Table 6. The mineral composition of the alcoholic extract of *T. bellirica* fruit.

Element	Composition (mg/kg)
Ca	50.72 ± 1.77
Mg	18.76 ± 0.12
Na	9.58 ± 0.03
K	4.26 ± 0.01
P	1.85 ± 0.02
Fe	7.59 ± 0.05
Mn	0.10 ± 0.01

Zn	0.13 ± 0.01
Pb	0.02 ± 0.01
Cd	0.01 ± 0.01
Na/K	0.51
Ca/P	6.68

Table 7. The comparative mineral composition of the alcoholic extracts of *T. bellirica* seed oil and seed kernel.

Element	Composition (mg/kg)	Composition (mg/kg)
Ca	120	300
Mg	20	50
Na	180	200
K	200	1,150
P	10	450
Fe	23	204
Mn	1	4
Zn	12	54
Cu	12	50

The values shown in Table 7 indicate that the *T. bellirica* exhibits a high nutritional value, and hence, it can be an essential supplement to living organisms. Further evidence clearly indicates that the alcoholic extracts of *T. bellirica* seed oil and seed kernel is highly suitable for edible purpose and it is most preferred in comparison to other seed oils.

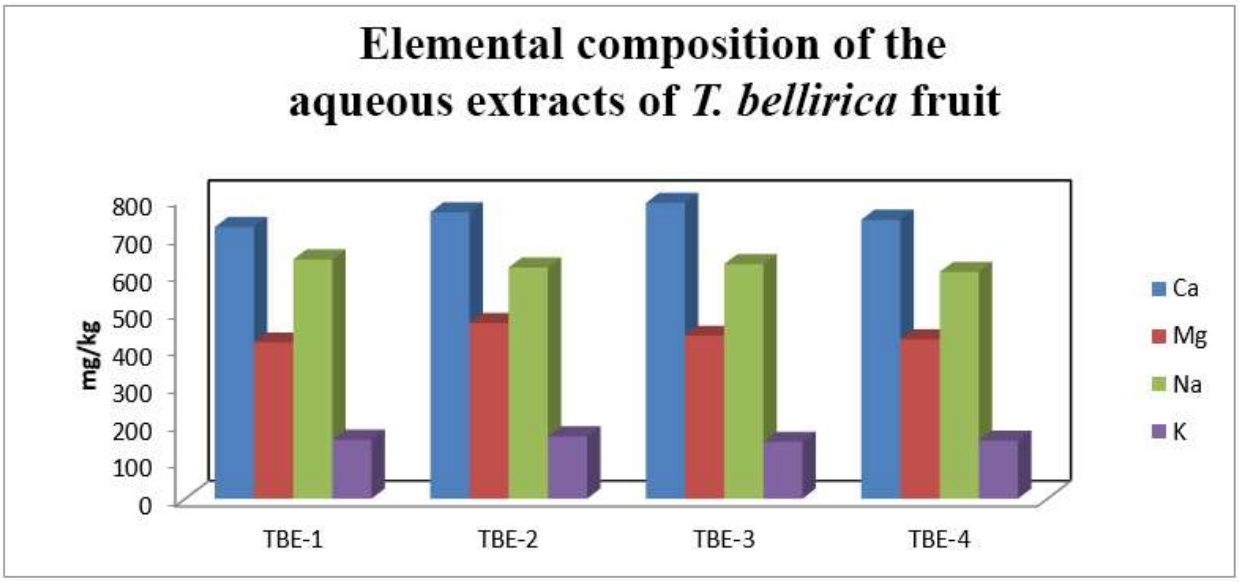

Figure 1. Elemental composition of the aqueous extracts of *T. bellirica* fruit (TBE-1, TBE-2, TBE-3, and TBE-4) [Ca, Na, Mg, and K].

Ca is the most abundant macro element in all tested *T. bellirica* extracts (Tables 4-7, Figure 1, and Figure 2). *T. bellirica* is one of the best bone healing medicinal herbs [26]. Ca is a vital element in building and maintaining strong bones and teeth. It is essential for blood clotting, maintenance of blood pressure and as a cofactor in enzymatic processes [27]. Proper use of Ca supplements with vitamin K2 as a promoter of bone and cardiovascular health [28]. The high concentration of Ca in *T. bellirica* fruit makes it a good source of nutrients for the elderly who are predisposed to osteoporosis since Ca provides rigidity to bones.

Ca plays an important role in many metabolic processes. Ca deficiency gives rise osteoporosis [29]. Ca content in the aqueous extracts of *T. bellirica* fruit is 754.05 mg/kg, 723.51 mg/kg (TBE-1) being the lowest amount and 787.39 mg/kg (TBE-3) being the highest amount (Table 4). Ca content in the finished products of *T. bellirica* is 404.40 mg/kg, 347.80 mg/kg (TBF-1) being the lowest amount and 478.90 mg/kg (TBF-2) being the highest amount (Table 5).

Ca content in the alcoholic extract of *T. bellirica* fruit was found to be 50.72 mg/kg (Table 6). Ca content in the alcoholic extracts of *T. bellirica* seed oil and seed kernel was found to be 300 mg/kg and 120 mg/kg, respectively (Table 7). Generally, not distinguished much variation between TBE-1 to TBE-4 but there is a noticeable amount of one of the formulation that is 193.50 mg/kg (TBF-2). In the alcoholic extract of *T. bellirica* fruit, Mg was found to be 18.76 mg/kg. Mg was found to be 20 mg/kg and 50 mg/kg in the alcoholic extracts of *T. bellirica* seed oil and seed kernel, respectively.

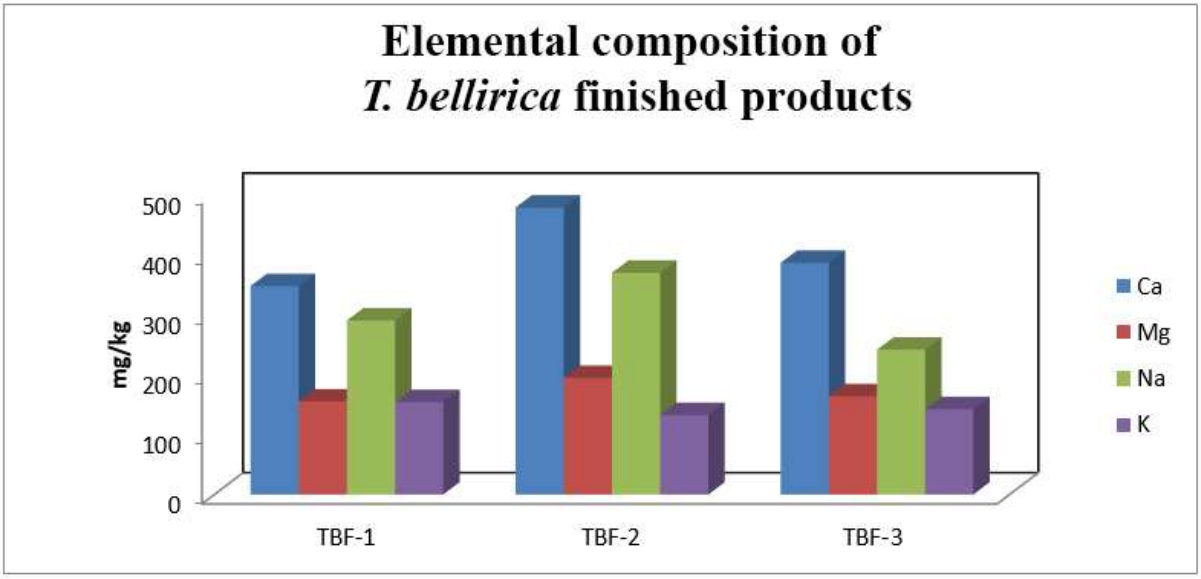

Figure 2. Elemental composition of the finished products of *T. bellirica* (TBF-1, TBF-2, and TBF-3) [Ca, Mg, Na, and K].

Mg has several biological functions in the human body including its role as a cofactor for more than 300 enzymatic reactions [30]. In clinical practice, optimizing Mg through diet and supplementation appears to be a safe, useful, and well-documented therapy for several medical conditions [31]. The values determined for the aqueous extracts of *T. bellirica* fruit are the best source for higher Mg content determination. Mg is a strongly electropositive, mobile, abundant divalent element for plants, with an average requirement for optimal plant growth in the range of 0.5-1.0% [32]. Mg is the third most abundant macro element in the aqueous extracts of *T. bellirica* fruit (Table 4 and Figure 1) and the finished products of *T. bellirica* (Table 5 and Figure 2). Mg content in the aqueous extracts of *T. bellirica* fruit was found to be in the range of 415.63-467.56 mg/kg (Table 4). Mg content in the finished products of *T. bellirica* was found to be in the range of 155.40-194.50 mg/kg (Table 5). Mg content in the alcoholic extract of *T. bellirica* fruit was found to be 18.76 mg/kg (Table 6). Mg content in the alcoholic extracts of *T. bellirica* seed oil and seed kernel was found to be 20 mg/kg and 50 mg/kg, respectively (Table 7).

Na and K levels in the aqueous extracts of *T. bellirica* leaves were reported to be higher than those in the aqueous extracts of *T. bellirica* fruits (62 mg/100 g and 15.7 mg/100 g, respectively) [33]. These macro elements are required for normal functioning of the nervous system and play a vital role in regulating blood pressure. It has been recommended that Na/K ratio less than 1.0 will prevent high blood pressure [34]. The Na/K-ATPase pump helps to maintain osmotic equilibrium and membrane potential in cells [35]. Our analysis shows that the Na/K ratio in the alcoholic extract of *T. bellirica* fruit was found to be 0.51 (Table 6), which is in conformity with the recommended ratio.

Na content in the aqueous extracts of *T. bellirica* fruit was found to be in the range of 603.45-636.79 mg/kg (Table 4). Na content in the finished products of *T. bellirica* was found to be in the range of 241.76-370.00 mg/kg (Table 5). Na content in the alcoholic extract of *T. bellirica* fruit was found to be 9.58 mg/kg (Table 6). Na content in the alcoholic extracts of *T. bellirica* seed oil and seed kernel was found to be 180 mg/kg and 200 mg/kg, respectively (Table 7).

K content in the aqueous extracts of *T. bellirica* fruit was found to be in the range of 151.23-165.73 mg/kg (Table 4). K content in the finished products of *T. bellirica* was found to be in the range of 132.70-154.40 mg/kg (Table 5). K content in the alcoholic extract of *T. bellirica* fruit was found to be 4.26 mg/kg (Table 6). K content in the alcoholic extracts of *T. bellirica* seed oil and seed kernel was found to be 200 mg/kg and 1,150 mg/kg, respectively (Table 7). Clearly, K content in the seed kernel is much higher than that in the seed oil.

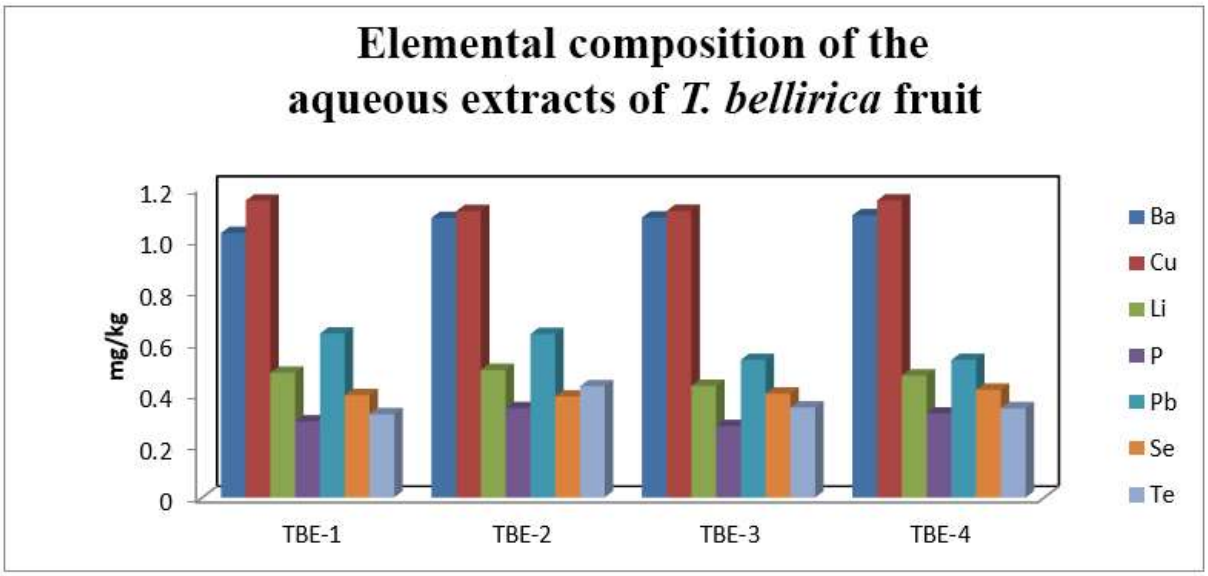

Figure 3. Elemental composition of the aqueous extracts of *T. bellirica* fruit (TBE-1, TBE-2, TBE-3, and TBE-4) [Ba, Cu, Li, P, Pb, Se and Te].

P is one of the most abundant minerals in the human body, second only to Ca [36]. This essential mineral is required for the healthy formation of bones and teeth, and is necessary for our bodies to process many of the foods that we eat. P is part of the body's energy storage system, and it helps maintain normal blood sugar levels [36]. The proper heart functioning, cell growth, and tissue repair processes require adequate amounts of P in the body [36]. P content in the aqueous extracts of *T. bellirica* fruit was found to be in the range of 0.2765-0.3457 mg/kg (Table 4). P content in the finished products of *T. bellirica* was found to be in the range of 0.1843-0.2015 mg/kg (Table 5). P content in the alcoholic extract of *T. bellirica* fruit was found to be 1.85 mg/kg (Table 6). P content in the alcoholic extracts of *T. bellirica* seed oil and seed kernel was found to be 10 mg/kg and 450 mg/kg, respectively (Table 7). Clearly, P content in the seed kernel is much higher than that in the seed oil.

The levels of Mg, P, Fe, Mn, and Zn in *T. bellirica* fruit are higher than those reported for other Combretaceae such as *T. Arjuna*[27] and *T. chebula* [37]. These elements are essential components of immune system and are vital for the synthesis of hemoglobin [27]. A food sample is considered to be valuable if the Ca/P ratio is greater than 1.0 and poor if it is less than 0.5 [38]. This is because a Ca/P ratio above 2.0 helps in the absorption of Ca in the small intestine. Moreover, the Ca/P ratio is as essential tool for the diagnosis of primary hyperparathyroidism and is of greater significance compared to serum Ca level.[38] As shown in Table 6, the Ca/P ratio in the alcoholic extract of *T. bellirica* fruit was found to be 6.68.

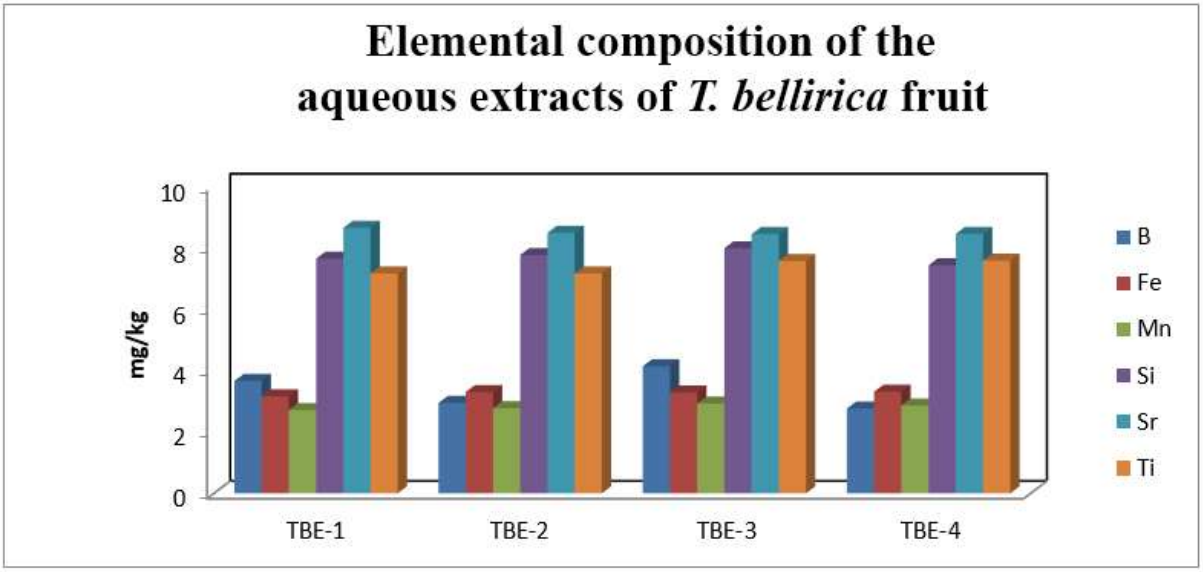

Figure 4. Elemental composition of the aqueous extracts of *T. bellirica* fruit (TBE-1, TBE-2, TBE-3, and TBE-4) [B, Fe, Mn, Si, Sr, and Ti].

The micro elements (B, Fe, Mn, Si, Sr, and Ti) are present in different amounts in the aqueous extracts of *T. bellirica* fruit (Table 4 and Figure 4) and the finished products of *T. bellirica* (Table 5). B is necessary for the brain to function properly and can increase mental alertness. Without small amounts of B, bones would slowly break down and become brittle [39]. B is a trace element capable of providing proper bone growth and development [40]. B positively influences minerals such as Ca, P, and Mg, and it acts in synergy with vitamin D [40]. B content in the aqueous extracts of *T. bellirica* fruit was found to be in the range of 2.76-3.65 mg/kg (Table 4). B content in the finished products of *T. bellirica* was found to be in the range of 1.25-1.38 mg/kg (Table 5). B content in the alcoholic extract of *T. bellirica* fruit and the alcoholic extracts of *T. bellirica* seed oil and seed kernel was below the detection limit.

Fe plays a critical role in oxygen binding to hemoglobin and in blood formation [41]. According to WHO (1997), two billion of the world's population are living with Fe-deficiency anemia. Fe is an indispensable constituent of heme-carrying proteins like cytochromes and Fe-S proteins [42]. Both in plants and animals, Fe plays an important role in electron transport system during cellular respiration [42]. Fe content in the aqueous extracts of *T. bellirica* fruit was found to be in the range of 3.1559-3.3074 mg/kg (Table 4). Fe content in the finished products of *T. bellirica* was found to be in the range of 1.3778-1.4676 mg/kg (Table 5). Fe content in the alcoholic extract of *T. bellirica* fruit was found to be 7.59 mg/kg (Table 6). Fe content in the alcoholic extracts of *T. bellirica* seed oil and seed kernel was found to be 23 mg/kg and 204 mg/kg, respectively (Table 7). Clearly, Fe content in the seed kernel is much higher than that in the seed oil. These findings suggest that supplementation of the diet with *T. bellirica* fruits can help in combating Fe-deficiency anemia and other related health problems.

Conclusion

For decades, plant extracts are analyzed to report on their therapeutic properties based on various organic molecules present in them. Here, an attempt was made to substantiate the claims of using *T. bellirica* extracts and finished products for their therapeutic values. In addition, our aim was also to assess the presence of phytochemical molecules like alkaloids, tannins, saponins, proteins, carbohydrates, organic acids, etc. We also evaluated the presence and content of various macro and micro elements in *T. bellirica* extracts and finished products. The elements of various metals could be used for treating certain common health conditions like diabetes (Cr, Mg, V, etc.), anemia (Fe), hair problems (Se), osteoporosis and other bone-related problems (Ca), as well as growth and overall health (Zn). Our data supplements the claims of using *T. bellirica* extracts and finished products for their therapeutic values in various medical conditions.

T. bellirica could be used as a daily dietary supplement as a preventive and rejuvenating agent. It is a highly recommended herb for a dietary supplement either alone or in combination (Triphala Churna). It is highly beneficial for overall health care and it is available in various forms and as an ingredient in many products. The high concentration of macronutrients such as Ca, Mg, Na, and K in the plant could be harnessed to combat nutrient deficiencies especially in the rural communities. *T. bellirica* is one the best bone healing herbs of choice due to having abundant quantities of Ca and Mg. The macro elements Na and K, which are required for normal functioning of the nervous system and play vital roles in regulating blood pressure, were also found to be abundant in all tested extracts of *T. bellirica*. The use of convectional medicinal drugs poses several adverse side effects on human health. Therefore, there is a need to incorporate edible phytochemicals into human diet as they are more effective, accessible, and inexpensive. The fruit samples of *T. bellirica* contain substantial amount of mineral elements. The elements analyzed in the present investigation will be helpful for drug prescription and at time of drug designing. The inorganic records evaluated in this study will be helpful to ensure the quality and verify adulteration of natural products in different ayurvedic formulations.

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