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Evaluation of The Physico-Chemical Characteristics, Minerals And Heavy Metals In Wild And Cultivated Variety Of *Aegle Marmelos* (L) Correa Leaves

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Abstract:

Background: *Aegle Marmelos* (L.) Correa belonging to family Rutaceae known in India from Vedic or prehistoric times is an important medicinal plant of India. There is wide genetic variability in terms of quality, form and size of the fruit. *Aegle marmelos* leaves are used as anti-diabetes agent in Ayurveda, Unani & Siddha Systems of Medicine

Materials and Methods: *Aegle Marmelos* leaves from the two varieties namely wild variety from Gir Somnath forest and newly cultivated variety "Goma yashi" were analysed chemically for proximate composition, available protein, fat, carbohydrates, fiber, mineral content and heavy metals. The values for the physico-chemical constituents and minerals is reported in percentage and heavy metals is reported in ppm.

Results: The wild variety was superior to the cultivated variety in terms of nutritional components like carbohydrates, protein, fiber, moisture content, ash content and many of the trace elements like Cu, Fe, Mn, Zn, Ca, Vanadium, Gold etc. However heavy metals like lead, arsenic, mercury and cadmium were not detected in either of the variety proving it to be non-toxic.

Conclusions: From the present study it is concluded that presence of various inorganic trace elements could account for the hypoglycaemic nature of the plant. Further, the data obtained on individual element concentration in the plant will be useful in deciding the dosage of herbal drugs prepared from this plant material for the management of diabetes-related metabolic disorders. The wild variety can further be exploited to isolate the bioactive constituents for its therapeutic claim.

Key words: *, physico-chemical, heavy metals, trace elements, hypoglycaemic*

Introduction:

Aegle Marmelos is a subtropical plant and grows up to an altitude of 1,200 m from sea level. It grows well in the dry forests, on hilly and plain areas. *Aegle Marmelos* is a widely distributed plant and found in India, Ceylon, China, Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia, Thailand, Indonesia, Malaysia, Tibet, Sri Lanka, Java, Philippines and Fiji. In India it found in Sub-Himalayan tracts from Jhelum eastwards to West Bengal, in central and south India. It found almost in all the states of India. It is generally cultivated in the vicinity of temple garden. It also vigorously grows in dry forest.[1] This tree is popular in '*Shiva*' and '*Visnu*' temples and it can be grown in every house. Its leaves are trifoliate symbolizing the '*Thrimurthies*'- *Brahma, Vishnu, Shiva*, with spear shaped leaflets resembling "*Thrisoolam*" the weapon of Lord *Shiva*. Many legends, stories and myths are associated with this tree. The leaflets are given to devotees as '*prasadam*' in *Shiva* temples and as '*Tulasi*' in *Vishnu* temples. [2] The bael fruit (*Aegle Marmelos* L. Correa) is known in India from Vedic or prehistoric times. Bael is the only member of the monotypic genus *Aegle*.

No drug has been longer and better known or more appreciated by the inhabitants of India than the bael fruits [3]. *Charaka* describes the plant as a *Rasayana*, besides its other actions and uses [4]. The importance of bael tree is highlighted in Yajur Veda, Buddhist and Jain literature - 'Upavana Vinod', 'Brihat Samhita' and

'Charaka Samhita', It withstands temperatures as low as -8^{0}C but under severe cold, it sheds its leaves. It is one of the choicest fruits of arid and semi-arid zones due to its drought resistence and tolerance to temperatures up to 48^{0}C. Its mature leaf emits a disagreeable odour when bruised. The plant leaves are found as effective ion-adsorbents of lead even in the presence of other heavy metal ions which makes the plant an excellent green alternate in control of pollution due to lead laden effluents from the industries.[5] The study indicated that the adsorption of Pb(II) occurs inside the wall of the hollow tubes present in the bael leaves and carboxylic acid, thioester and sulphonamide groups are involved in the process. Bael leaves could selectively remove Pb(II) in the presence of other metal ions was demonstrated by removing Pb from the effluent of exhausted batteries.

Aegle Marmelos (L.) Corr. belongs to the family Rutaceae. The common names for the tree are Bael in Hindi, Bilwa in Sanskrit, Maredu in Telugu, Bil in Gujarati, Kumbala in Kannada, Vilvam in Tamil and Kuvalam in Malayalam. Kumbala in Kannada, Vilvam in Tamil and Kuvalam in Malayalam. The leaves and the shoot of the plant are used as green vegetable in Indonesia.[6-7] Biochemical compounds of bael leaves, fruits and seeds have been used in several diseases like diabetes, cardiovascular and anti-inflammatory.[8] Aegeline (N-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide) is a known constituent of the bael leaf and consumed as a dietary supplement for a variety of purposes.[9-11] The most important ingredients present in plants are alkaloids, terpenoids, steriods, phenols glycosides and tannins.[12] The bael leaf contain seven monotorpene hydrocarbons (90.7%), three oxygenated monoterpenes (2.9%), four sesquiterpene hydrocarbons (3.1%) and one phenolic compound (0.2%). Limonene (82.4%) was the main constituent of bael. [13] *Aegle Marmelos* leaf extract (200 mg/dl for 35 days) significantly affect the activity of lipid peroxidase, lipoprotein and antioxidant enzymes in isoproterenol treated rat.[14]

Aegle Marmelos Leaf extract was effective in restoring blood glucose, body weight to normal values and significantly reversed the altered (histological and ultra-structural) parameters in tissues of streptozotocin induced diabetic rats seen by light and electron microscopy to near normal and improved the functional state of pancreatic beta cells. [15]
The hypoglycemic effects of this plant drug appear to be mediated through regeneration of damaged pancreas.[16] Bael leaf enhances ability to utilize the external glucose load in the body by stimulation of glucose uptake similar to insulin. Bael extract significantly lowers blood urea, reduction in lipid peroxidation and cholesterol and increased levels of super dioxide dismutase, catalase, glutathione peroxidase and glutathione level in serum as well as in liver in experimental diabetic animals. [17] Bael leaves taken every morning reduce blood pressure due to presence of potassium which maintains dilation of blood vessels. [18] Aegeline 2, a bioactive compound present in leaves of have antihyperglycemic and hyperilipidemic activity as evidenced by lowering the blood glucose levels, decreased the plasma triglyceride, total cholesterol and free fatty acids accompanied with increase in HDL-C and HDL-C/TC ratio.[19] Clinically bael leaves also have shown antidiabetic activity in NIDDM subjects.[20] Bael leaves is used in management of hyperglycaemia where the sugar level in blood and urine reduced significantly by the end of 2 months. [21]

These days great attention is being given to management of diabetes with medicinal plants along with dietary restriction. Modern medicine is rooted in ethno botanical traditions using indigenous flora to treat symptoms of human diseases or to improve specific aspects of the body conditions. Today a great number of modern drugs are still derived from natural sources and 25 per cent of all prescriptions contain one or more active ingredients from plants.[22] Medicinal plants are inextricably interwoven with the rich history, culture, and culinary tradition of India. It is reported that 4639 ethnic communities living in different regions of India use locally available traditional medicinal plants to treat various ailments. Medicinal plants are also used by the codified systems of medicine such as Ayurveda, Siddha and Unani. *Aegle Marmelos* is one of the 573 Medicinal plants used commonly in Ayurveda, Unani & Siddha Systems of Medicine In most preparations, the medicinal plants being used very often are in powder or paste forms of the crude herbs containing both organic and inorganic constituents. Various plants are used to manage diabetes, such as *Acacia Arabica, Annona squamosal, Camellia sinensis, Capsicum frutescens, Cinnamomum zeylanicum, Ficus religiosa, Zingiber officinale,* , etc.[23] WHO has estimated that 80 per cent of the population of developing countries still relies on traditional medicines mostly plant drugs for their primary health care needs and ensure patient safety by upgrading the skills and knowledge of traditional healers. [24]

It is known that certain inorganic trace elements such as vanadium, Zinc chromium, copper, iron, potassium, sodium and nickel play an important role in the malignance of normoglycemia by activating the beta-cells of the pancreas. Research during the past two decades has added chromium, nickel, vanadium, selenium, silicon, molybdenum, iron, and arsenic to the list of essential elements. Plants are an important medium in the transfer of trace elements from soil to humans. [25]
Important studies have found a significant modulatory role of trace elements in various diseases [26-27]. It has been documented that alteration of trace elemental homeostasis in an organism has direct correlation with different pathological conditions.[28] However, they may be toxic if consumed beyond their estimated safe daily intake. Several works have been reported on the phytochemical and biological activities of medicinal plants, although there is little report in regards to the heavy metal contents of these plants. Medicinal herbs can present health risks due to the presence of toxic metals such as Pb, Cd, Al, and Hg which are hazardous to humans. *Aegle Marmelos* plant has been included in the WHO list of herbal drugs wherein HPTLC fingerprint is available for standardization of the herb [31] The Ayurvedic Pharmacopoeia of India includes the monographs on "stem" ad "root" of the plant. However reports on physico-chemical constituents and trace elements present in the leaves are few though this plant has been regarded as good dietary supplement. [32-33] However for a clear picture of nutritional status of varieties being cultivated in India, a systematic work needs to be carried out.

The present investigation is an attempt to gain an insight into the physico-chemical, trace elemental composition of an important medicinal plant *Aegle marmelos* from Gujarat state of India.

Ayurveda, ancient system of Indian medicine describes two varieties of bael- wild that grows in forest and the cultivated variety which is grown near the temples throughout India. There is wide genetic variability in terms of quality, form and size of the fruit [34] There are no standardized names for bael cultivars. They are named after the locality where they are most easily available. Its few main cultivated varieties in India are Mirzapuri, Kagzi, Gonda, Kagzi Banarsi, Kagzi Etawah, Narendra Bael-1, Narendra Bael-2, Narendra Bael-5 and Narendra Bael- 9, Pant Urvashi, Pant Shivani, Pant Aparna, Pant Sujata, CISH-B1 and B-2, etc. which are based on size of fruits, amount of pulp, TSS, amount of fibres in fruit, average yield of fruits per plant, thickness of fruit rind, acidity, seeds etc.[35] However they are all cultivated variety or genotype of *Aegle marmelos*. The wild forms of *Aegle marmelos* have small fruits (5 to 7.5 cm in diameter), whereas in cultivated forms the fruits may attain a diameter of 12.5 to 17.5 cm. Gir Somnath, in Gujarat happens to be the first *Jyotirlingham* among the twelve *Jyotirlinghams* of India. It has a famous Somanth temple of Shiva and good amount and quality of wild variety of *Aegle marmelos* grows in the forest of Sasan Gir. *Aegle marmelos* leaves are offered in prayers to deities Lord Shiva and thus the tree is also known as Shivdruma (The tree of Shiva).

The studies reporting on the differences in physico-chemical characteristics in leaves in different genotypes as well as in wild and cultivated are very few as per our knowledge. In fruit crop like bael where the differences in growth rate and productivity are quite apparent, studies into variations of leaf nutrient content assume special significance. In view of the above mentioned fact, the present investigation was carried out to compare the physico-chemical characteristics, mineral content and heavy metal content of wild variety of Gir forest and newly cultivated variety "Goma yashi" from Central Horticultural Experiment Station (CIAH), Vejalpur (Godhra), Panchmahal, Gujarat in India.

METHODOLOGY

Collection of Plant Material and identification

Sample for the cultivated variety of *Aegle Marmelos*, a newly developed variety named "Goma yashi" was procured from Central Horticultural Experiment Station (CIAH), Vejalpur (Godhra), Panchmahal – 389340. Central Horticultural Experiment Station (CHES) in Godhra has developed a new variety of bael, the indigenous dry land fruit tree of the country. The new spineless bael variety - 'Goma Yashi' - has been named after river Goma, which passes through the tribal-dominated Panchmahal district where CHES, the research station working under Bikaner-based Central Institute for Arid Horticulture (CIAH) is based. CIAH works under the aegis of New Delhi-based Indian Council of Agricultural Research. Sample for the wild variety was collected from the identified zone of Gir forest ($21^0$04'49.7"N $70^0$35'10.6"E). Fresh leaves were thoroughly washed to remove unwanted material and dirt and then dried under shade. They were then dipped in 1%$HgCl_2$ solution for a minute and then dried on a filter paper. They were then identified and authenticated by Dr. Padmanabhi Nagar, Associate Professor of the department of Botany, M.S. University of Baroda, Vadodara. A voucher specimen of the leaves (A.M./FND/10) was prepared and preserved in the department of herbarium.

Preparation of the plant material for testing:

The fresh good quality of leaves from both the variety were washed, cleaned and dried under the shade for 2-3 days until brittle. Dried leaves were then powdered to pass through 40 mesh size. The leaves were then stored in air tight plastic container for further use.

Bael leaves were analysed chemically for proximate composition- total ash and available moisture and mineral content [36], available carbohydrates [37] (with some modifications), dietary fiber (by FIBER THERM), protein [38], fat content [39] and heavy metal analysis by Atomic Absorption Spectrophotometer (Thermo scientific).

Ash

2-4 grams of the sample was weighed accurately in a previously ignited and tarred silica dish. The material was spread evenly and ignited in a muffle furnace at 600^0C until it was white, indicating the absence of carbon. The dish was cooled in a desiccator and weighed. The percentage of the total ash of the air-dried material was calculated.

Calculation

$$\text{Total ash (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of the sample taken}}$$

Moisture

Moisture content of leaves were determined by drying the weighed sample of leaf at 105^0C in hot air oven for 5 hours and the loss of weight was expressed as moisture content. Five gram leaf sample from each variety was taken in pre-weighed petri plates and the moisture was calculated by the following formula:

$$\textbf{Moisture (\%)} = \frac{\textbf{(Fresh weight} - \textbf{Dry weight)}}{\textbf{Fresh weight}} \textbf{ X 100}$$

Total carbohydrate

The leaf 1 g was homogenized in 2N hydrochloric acid using mortar and pestle and volume was made to 20 ml. The content was refluxed for one hour on boiling water bath at 70˚C. Supernatant was collected and residue was re-extracted twice with 2N HCl. All supernatant were pooled and final volume was made to 50 ml. The extract was used for the estimation of total carbohydrates. Total carbohydrates were estimated with some modifications. Aliquot 0.5 ml was taken and volume was made to 3 ml with the distilled water followed by 0.5 ml distilled phenol and mixed thoroughly. To this, 5.0 ml concentrated sulphuric acid was carefully added at the side of the tube. After the mixing thoroughly the tubes were kept for 30 minutes at room temperature for colour development. The absorbance was measured at 490 nm.

$$\textbf{Carbohydrate (\%)} = \textbf{Graph factor x} \frac{\textbf{Sample reading}}{\textbf{weight of sample}} \textbf{x} \frac{\textbf{Total volume}}{\textbf{Taken volume}} \textbf{x } 10^{-4}$$

Protein

The leaf sample 1 gram was weighed and homogenized in five ml 0.1 N NaOH and filtered through Whatman No.1 filter paper. The sample extracts 0.2 ml was taken and made to 3.0 ml volume with distilled water. 5 ml of alkaline copper solution (50 ml 2% Na_2CO_3 in 0.1 N NaOH + 1 ml 0.5 % $CuSO_4$ in 10 % Sodium Potassium tartrate) was added. The content was allowed to stand for 10 minutes at room temperature followed by addition of 0.5 ml solution Folin Ciocalteu reagent (1:1 v/v). The content was kept for 30 minutes at room temperature and the absorbance was measured at 750 nm. The protein content was calculated using bovine serum albumin as standard range from 50 – 300 µg.

$$\textbf{True protein (\%)} = \textbf{Graph factor x} \frac{\textbf{Sample reading}}{\textbf{weight of sample}} \textbf{x} \frac{\textbf{Total volume}}{\textbf{Taken volume}} \textbf{x } 10^{-4}$$

Fiber

Fiber extraction was done by FIBER THERM

Fiber Extraction

First sample weight (1gm) was taken W1. After washing in fiber therm the sample was dried at 105^0C for 3-4 hours. After drying in oven and cooled in desiccator for 30 min, the sample was transferred to crucible (weight of crucible - W0). Weight of crucible with dried sample W2 was taken. Now samples were kept into muffle furnace at 600^0C for 30 min and cooled to room temperature in desiccators. Then the weight of crucible along with ash was taken, assuming it as W3

$$\textbf{Fiber (\%)} = \frac{\textbf{(W2-W0) - (W3-W0)}}{\textbf{W1}} \textbf{ X 100}$$

Note :- wash the crucible with Acid H_2SO_4 0.313 and then NaOH 0.255N.

Fat

Fat content was determined by the soxhlet method. Three to four grams of sample was taken in a thimble and it was placed in soxhlet apparatus fitted with a condenser. 100 ml of Petroleum ether (B.P. 40-60^0 C) in the round bottom flask and boiled for 4 hours. The extract was taken in pre-weighed conical flask. The conical flask was kept in a water bath to evaporate the petroleum ether. The traces of petroleum ether were removed in vacuum pump. The weight of fat was taken to constant weight.

Calculation

$$\textbf{Fat content (\%)} = \frac{\textbf{Weight of petroleum ether extract} \times \textbf{100}}{\textbf{Weight of the sample taken}}$$

Heavy Metal Analysis

Digestion of the Sample

Leaf samples were digested in 1:2 Sulphuric acid and Nitric acid (30gm sample in 30ml acid solution).

Heavy metal estimation

It was done by (AAS) Atomic Absorption Spectrophotometer (Thermo scientific)

The digested contents were transferred to acid washed bottles and made up to 100ml with double distilled water and subjected to various metal analysis in Atomic Absorption Spectrophotometer (Thermo scientific). The trace metals in the digested samples were determined by Atomic Absorption Spectrophotometer with either air-acetylene flame, a standard mercury analyser (ECIL) was used for Mercury estimation by cold vapour Atomic Absorption Spectrophotometer following the AOAC method. The results were expressed in ppm.

Results:

The wild and the cultivated variety of *Aegle marmelos* leaves were analysed for ash, moisture, protein, carbohydrates, fat, fibre total phenols, Minerals and heavy metals. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. Ash usually represents the inorganic part of the plant [40] Nutritionally fiber is beneficial to the human body since it has been reported that food fiber aids absorption of trace elements in the gut and reduce absorption of cholesterol. [41] Fiber aids bowel movements of the gut.[42] The fibers provide synergistic protection for colon cells from free radical damage to their DNA. The ash, moisture, protein, carbohydrate, fat and crude fiber content of wild variety of Gir and cultivated variety called *Goma yashi* were 6.5% and 6%; 53.96% and 52.14%; 7.6% and 2.22% ; 10.38% and 5.42%; 8.18% and 12.78% and 30.14% and 25.10% respectively

Various studies reported the results in this manner; *Aegle marmelos* leaf had 10.3 g ash.[43] Another study reported that the*Aegle marmelos* leaf had 1.8±0.10g of crude fat, 14.8±0.13g of crude fiber, 9.2±0.03g of ash, 1.8±0.09g of carbohydrate ,66.5±0.46 g of moisture, 5.9±0.12g of crude protein.[44]

A study reported ash, carbohydrate, fat, protein, fiber content in to be 8.6g/100g, 10.5±0.3g/100g, 1.7±0.5g/100g, 6.2±0.2g/100g and 18g/100g respectively.[45]. In a study ash content in *Aegle marmelos* leaf was analysed as per WHO guidelines and was reported it to be 9.0% whereas another investigator reported ash content to be 6.3% which is similar to our result and moisture content to be 67% respectively [46-47]. The proximate composition of *Aegle marmelos* leaves of wild and cultivated variety is given in table 1 and Figure1.

It is known that certain inorganic trace elements such as vanadium zinc, chromium, copper, iron, potassium, sodium, and nickel play an important role in the maintenance of normoglycemia by activating the β-cells of the pancreas. In the present study, the elemental composition in the leaves of two varieties of *Aegle marmelos* traditional medicinal plant- wild one from Gir forest and cultivated variety called "*Goma yashi*" widely used in the treatment of diabetes-related metabolic disorders has been studied using atomic absorption spectroscopy. The levels of Magnesium for wild variety and cultivated variety were 6.3% and 6.5% respectively; Silicon 3.9% and 4.03% respectively; Chlorine 4.0% and 5.68% respectively; Potassium 2.70% and 2.46% respectively; calcium 7.9% and 7.6% respectively; Titenium 0.057% and 0.099% respectively; Vanadium 0.043% and in cultivated variety it was not respectively detected. Chromium content for the wild and cultivated variety was 0.067% and 0.026%; Nickel 0.14% and 0.049% respectively; Bromium 0.374% and 0.547% respectively; Strontium 0.693% and 0.719% respectively; Barium 0.24% and 0.25% respectively; Rhenium 0.04% for both the varieties. Aurum (Gold) content was also detected for both the varieties (0.11% and 0.095% for wild and cultivated variety respectively). Trace elements in both the varieties are given in table 2 and 3 and figure 2, figure 3 and figure 4.

Another investigator analyzed the trace elements in *Aegle marmelos* leaves by Atomic Absorption Spectroscopy (AAS) and reported the trace elements in *Aegle marmelos* leaves having Cr, Cu, Zn, V, Fe, and Ni to be 1.73µg, 0.38 µg, 0.14 µg, 0.24 µg, 2.67 µg and 0.81 µg in 1gram of ash respectively. [47]

Few investigators evaluated macro-elements and trace elements in ten edible medicinal plants and found large variations in the concentration of metals in plants samples studied. Out of the total contents of metals in these plants, the macro-metals including Cd (0.00024-0.00028%), Pb (0.00032-0.00047%), Mg (0.20-0.54%), Ca (0.32-0.39%), and Al (0.008-1.64%) were present in very low concentrations whereas Na (18.76-38.95%) and K (59.66-78.67%) were present comparatively in very high concentrations. Similarly, the trace elements, Cu (0.0022-0.016%). Cr (0.16-0.44%) and Ni (0.38-0.66%) showed the lowest concentration and Mn (2.59-14.50%) and Fe (84.36-96.87%) showed the highest concentration which may be toxic to health.[48]

Aegle Marmelos is an important traditional plant. The ash value, heavy metals, pesticide residue is important to check contamination and toxicity. Heavy metals were analysed through Atomic Absorption Spectrophotometer (AAS) and none of the metals like Cadmium, Arsenic, Lead and Mercury were detected in our study for both the varieties. The heavy metal analysis of the two varieties is given in table 3.

A study was done in which *Aegle marmelos* leaves were analysed for heavy metals, pesticide residues and microbial contamination as per the WHO guidelines and found the heavy metals like Lead and Cadmium to be 2.86ppm and 0.09ppm within permissible limits while Mercury and Arsenic and Total pesticide residues were not detectable (ND). [49]

Some of the commonly used traditional medicinal plants in Ghana were analysed for their heavy metal toxicity and sample locations by Atomic Absorption Spectrometer using wet digestion. The result indicated that Pb(II) was present in all plant species examined, except *Ocimum gratissimum*. Significant variation existed in mineral content for the various locations ($P \leq 0.05$). The findings generally suggested the variation in mineral levels for the various locations. It was shown that the same species of medicinal plants, growing in different environments, accumulates different levels of heavy metals. [50]

Table 1: Proximate composition of the two varieties

Sr. No.	**Parameter**	***Aegle marmelos* plant (wild)- Gir forest (%)**	**Newly developed *Aegle marmelos* variety '*Gomayasi*' (%)**
1	Total Ash	6.5	6.0
2	Moisture	53.96	52.14
3	Protein	7.60	2.22
4	Carbohydrate	10.38	5.42
5	Fat	8.18	12.78
6	Fibre	30.14	25.10

Table 2: Mineral Content of the two varieties

Sr. No.	**Minerals**	***Aegle marmelos* plant (wild)- Gir forest (ppm)**	**Newly developed *Aegle marmelos* variety '*Gomayasi*' (ppm)**
1	Copper	12	11
2	Iron	181	165
3	Manganese	62	56
4	Zinc	49	38

Table 3: Trace elements of the two varieties

Sr.No	Parameter (%)	*Aegle marmelos* plant (wild)-Gir forest (%)	Newly developed *Aegle marmelos* variety "*Gomayasi*" (%)
1	Magnesium	6.3	6.5
2	Silicon	3.9	4.03
3	Sulphur	0.92	1.51
4	Chlorine	4	5.68
5	Potassium	2.7	2.47
6	Calcium	79.29	76.88
7	Vanadium	0.043	0
8	Chromium	0.067	0.026
9	Manganese	0.492	0.07
10	Nickel	0.14	0.049
11	Bromium	0.374	0.547
12	Strontium	0.693	0.719
13	Barium	0.24	0.25
14	Rhenium	0.04	0.04
15	Aurum (gold)	0.11	0.095

Table 4: Heavy metal content of the two varieties

Sr. No.	Heavy metal (ppm)	*Aegle marmelos* (wild variety)-Gir forest	Newly developed *Aegle marmelos* (cultivated variety) "*Gomayasi*"
1	Cadmium	Nil	Nil
2	Arsenic	Nil	Nil
3	Lead	Nil	Nil
4	Mercury	Nil	Nil

Discussions

It is known that certain inorganic elements such as V, Zn, Cr, Cu, Fe, Na, K, and Ni play an important role in the maintenance of normoglycaemia by activating the β cells of the pancreas. In this study, the elemental composition in the leaves of *Aegle marmelos* widely used in the treatment of diabetes-related metabolic disorders has been studied using atomic absorption spectroscopy. The levels of Cu, Ni, Zn, K, Na Fe, Cr, and V were found to be in trace amounts. This study demonstrated that the trace metal content in *Aegle marmelos* leaves have potential antidiabetic activity. The knowledge of the chemical form of the elements in plants of economic interest might be crucial because actions can be taken to reduce or minimize the toxic effects of the environment pollutant heavy metals.[51]

The yields of the ash were relatively higher than the total organic extracts. The role of inorganic elements like Zn, Cr, V, Fe, Cu, and Ni in the improvement of impaired glucose tolerance and their indirect role in management of diabetes mellitus are being increasingly recognized [52-53]. Vanadium, a group VB transition element, was effective in lowering glucose and had cardio protective effect in streptozotocin (STZ) diabetic rats [54]. Insulin mimetic actions of vanadium have been well documented in several in vitro and in vivo models of Type I diabetes, including chemically STZ-induced diabetes in rats improving glucose homeostasis in generally obese, hyperinsulinemic, and insulin-resistant rats showing some of the characteristics of Type II diabetes [55-56]

Complexes of zinc and insulin in varying proportion are stored in pancreatic β -cells and released into the circulation via the portal vein.[57] Abnormal zinc metabolism has been suggested to play a role in the pathogenesis of diabetes and its complications and tissue zinc deficiency has been observed in genetically obese, insulin-resistant diabetic mice.[58]

Zinc deficiency in diabetic conditions appeared to result from hypo-glycaemia, impaired zinc absorption, and excessive zinc excretion. Zinc enhances the effectiveness of insulin in vitro. [59] However the clinical significance and evaluation of zinc in regard to different diseases, including diabetes remains conflicting as well as controversial. It may act in normalizing glycaemia and a restored zinc status in patients with type 2 diabetes mellitus may counteract the deleterious effects of oxidative stress, thereby preventing many complications associated with diabetes.[60] Improvement of glycaemic control and supplementation with zinc appears to be a beneficial factor in decreasing lipid peroxidation in patients with diabetes. Prevention of lipid peroxidation may help to delay the development of diabetic complications.
Following Zinc and iron, copper is the third most abundant element in the body. It is involved in the oxidation of Fe^{2+} to Fe^{3+} during haemoglobin formation. It is important catalyst for iron absorption. Copper deficiency may be a risk factor for cardiovascular disease neutropenia, osteoporosis and anaemia. [61] Excess copper is toxic.
Iron is essential for human body for haemoglobin production in the body and for oxygenation of red blood cells. It is needed for healthy immune system and energy production. Severe iron deficiency leads to anaemia and red blood cells that have low haemoglobin concentration. In children it results in reduced cognitive performance, reduced attention and slow growth. In adults iron deficiency affects physical work performance.

Chromium, a group VIB transition element, is important for normal carbohydrate and lipid metabolism [62]. It is essential as for working as a glucose tolerance factor. Deficiency of chromium has been implicated as one of the causes of diabetes mellitus. [63-64]

For Nickel, urease, the Nickel containing enzyme is used for hydrolysis of urea. The functional value of Manganese are as a Lewis acid and catalyst for oxidation. It is essential to all organism, activates numerous enzymes. Selenium is constituent of glutathione peroxidase and other enzymes and has antioxidant property. The deficiency of it may lead to muscle and pancreatic degeneration haemolysis. Arsenic takes a role in metabolism of methyl compounds and the deficiency of it will lead to impairment of growth reproduction and heart function. The function of Titanium is not known yet. It is harmless to our body. Bromine may be essential too in mammals. It is non-toxic except in oxidizing forms. The function of Rhenium and Strontium is unknown.

Normal potassium concentration is necessary for optimal insulin secretion, and deficiency of potassium causes diabetic acidosis. Potassium depletion can result in impaired glucose tolerance. Potassium ion play an important role in the diseases related to renal disorders. [65]

Enzymes that do not contain a trace element as an integral part but are activated by metals such as Cu, Fe, and Ni respond to in vitro addition of several transition elements with a dose-dependent activation. [66] This suggests that the high degree of specificity in vivo is brought about by carriers with specific sites that identify certain element when it enters the organism and delivers it to its own site of action, but not other site. The carrier substances assure the delivery of trace elements to their specific sites of action. At these sites, the action of trace element is specific and is dependent on properties such as valence state, redox potential, ionic radius, coordination number, coordination geometry, spin state (high slow spin transition), and rate of ligand exchange.[67]

From the present study, it is concluded that the presence of various inorganic trace elements such as vanadium, zinc, chromium, copper, iron, nickel, potassium, and magnesium in the leaves of *Aegle marmelos* could account for the hypoglycaemic nature of the plants. Further, the data obtained on individual element concentration in each plant will be useful in deciding the dosage of herbal drugs prepared from these plant material for the management of diabetes related metabolic disorders. Ongoing studies are progressing in evaluating the mechanism(s) of action of these trace elements present in the ash, in lowering the blood glucose level in experimental diabetes.

Some of the daily requirement of these elements are as follows; Iron 100mg per day for male, 15mg for female per day; Zinc 15mg/day, Mn 2.5-5mg/day, copper 2-3mg/day; chromium and Selenium -9.95-0.2mg/day.

The non-detection of heavy metals leads to the fact that these plants are grown in pollution free areas of such elements and elemental uptake by these plants depends on soil characteristics and climatic condition. It is also an important environmental protector as leaves act as a sink by absorbing dust and foul and poisonous gases from surrounding atmosphere and makes them clean. [68]. Owing to its environment friendly nature, is being placed among plant species group called “climate purifiers” which emit a greater percentage of oxygen in sunlight as compared to other plants.[69] Due to its endless uses, is also known as *Mahaphala* or Great fruit.[70]

Conclusions:

Two varieties/accessions of *Aegle marmelos*-Wild variety from Gir Somnath and a newly developed cultivated variety from Vejalpur Research Station was evaluated for its physico-chemical properties, mineral elements and heavy metals. Moisture, Protein, fiber and carbohydrate content were more in Wild variety in comparison to the cultivated variety. Important minerals like copper, Iron, Manganese, Zinc Potassium, calcium, Vanadium, Chromium, Nickel and aurum (gold) content were more in the wild variety as compared to the cultivated variety. Whereas magnesium, Silicon, sulphur, chlorine, titanium, bromium, Strontium and Barium content was more in cultivated variety. As far as heavy metals are concerned, they were not detected in either of the varieties which show that *Aegle marmelos* leaves were non-toxic. This highlights its important therapeutic role as anti-diabetic plant. The wild variety seemed to be superior in nutritive value and trace elements in comparison to the cultivated variety “*Gomayasi*”. The plant studied here can be seen as a potential source of useful drugs. Further studies are going on in order to identify, characterize and elucidate the structure of the bioactive compounds.

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Conflict of Interest:

The author wishes to declare that there is no conflict of interest.

References:

1. Dhankhar, S. Ruhil, M. Balhara, S. Dhankhar, A.K. Chhillar, Journal of Medicinal Plants Research, 2011, 5, 1497-1507.
2. Roy S.K. and Singh R.N. Studies on utilization of bael fruit for processing II Extraction of Bael fruit pulp Indian Food Pac. 1979; 33(1); 5-9
3. Chopra R.N.; Indigenous Drugs of India; Academic Publishers, Calcutta (1982)
4. Charaka-Samhita, (Ed.) G.S. Pandeya, Chowkhamba Sanakrit Sansthan, Varanasi, (1983)
5. Chakravarty S. Mohanty A, Sudha TN, Upadhyay AK, Konar J, SircarJk et al.Removal of Pb (II) ions from aqueous solution by adsorption using bael leaves (*Aegle marmelos*). J Haz Mat 2010, 173;502-509
6. P.C. Sharma, v. Bhatia, N. Bansal, A. Sharma, Natural Product Radiance, 2007, 6, 171-178.
7. Rathore M., Journal of Horticulture and Forestry, 2009, 1, 103-108.

8. Maity P., Hansda D., Bandopadhyay U. and Mishra D.K. Biological activities of Crude extracts and chemical constituents of Bael, *Aegle marmelos* (L.) Corr.2009. Indian Journal of Experimental Biology, 2009; vol 47,(nov); pp 849-861.
9. Govindachari TR, Premila MS. Some alkaloids from *Aegle marmelos*. Phytochem.1983; 22: 755.
10. Riyanto, S; Sukari MA; Rahmani M; et al."Alkaloids from *Aegle marmelos* (Rutacea).". Malaysian J Anal Sci.2001 7 **2**: 463–465.
11. Lanjhiyana, S; Patra KC; Ahirwar D; et al. "A validated HPTLC method for simultaneous estimation of two marker compounds in *Aegle marmelos* (L.) Corr., (Rutaceae) root bark.". Der Pharm Lett. 2012 4 1: 92–97.
12. Venkatesan D, Karrunakarn CM, Selva SK and Palani PTS. Identification of Phytochemical Constituents of *Aegle marmelos* Respon sible for Antimicrobial Activity against Selected Pathogenic Organisms. Ethnobotanical Leaflets 2009;13: 1362-72.
13. Kaur HP, Garg SN, Sashidaran KV, Yadav A. Chemical composition of essential oil of the twigs and leaves of *Aegle marmelos* (L.) Correa. J Essen Oil Res. 2006; 16: 144-145.
14. Rajadurai H, Prince PSM. Comparative effects of *Aegle marmelos* extract and α-tocopherol on serum lipids, lipid peroxidase and cardiac enzymes levels in rats with Isoproterenol induced myocardial infarction. Singapore Med J. 2005; 46(2): 78.
15. Upadhya S, Shanbagh KK, Sunneetha G, Balachandra Naidu M. A study of hypo glycaemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. Indian J Phsiol Pharmacol. 2004; 48(4): 476-480.
16. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol. 2000; 32: S81-S118.
17. Sharma PC, Bhatia V, Bansal N and Sharma A. A review on Bael Tree. Natural Product Radiance 2007 6(2): 171-178.
18. Parichha S. Bael (*Aegle marmelos*) Nature's Most Natural Medicinal Fruit. Orissa Review, 2004; 16-17.
19. Narender T, Shweta S, Tiwari R, Papi Reddy K, Khaliq T, Prathipati P, Puri A, Srivastava AK, Chander R, Aggarwal SC, Raj K. Anti hyperglycemic and anti dyslipidemic agent from *Aegle marmelos*. Bioorg Med Chem Lett. 2007; 17(6):1808-1811.
20. Yaheya M and Ismail M. Clinical Evaluation of Antidiabetic Activity of Bael Leaves. World Applied Sci J 2009. 6(11): 1518-1520.
21. Narendhirakannan R.T, Subramanian S, and Kandaswamy M. Mineral Content of Some Medicinal Plants Used in the Treatment of Diabetes Mellitus. Biological Trace Element ResearchVol. 2005;103
22. Shankhla A, Sharma S and Sharma N. Hypoglycemic effect of bael patra(*Aegle marmelos*) in NIDDM patients. J Dairying, 2009; Food and Home sci 28: 3-4.
23. Mandal PK, Mukherjee AK. Investigation on partial structure of a glycoprotein from bael seed. Carb Res.1981; 98(1): 85-91.
24. Thorfeldt S. Herbs in diabetes mellitus. Alt Med Rev 2005. 9 ;13-18.
25. Sachdewa A, Raina D, Srivastava AK, Khemani LD. Effect of *Aegle marmelos* and Hibiscus rosa sinensis leaf extract on glucose tolerance in glucose induced hyperglycemic rats (Charles foster). J Environ Biol. 2001; 22(1): 53-57.
26. Bhushan MS, Rao CHV, Ojha SK, Vijayakumar M, Verma A. An analytical review of plants for anti-diabetic activity with their phytoconstituent & mechanism of action. Int J Pharm Sci Res. 2010;1(1):29–46.
27. Ponnachan PTC, Paulose CS, Panikkar KR. Hypoglycaemic effect of alkaloid preparation from leaves of *Aegle marmelos*. Amala Res Bull. 1993; 13: 37–41.
28. WHO 2008. World health Statistics (http://www.who.int/whosis /whostat /2008 /en/)
29. K. Wierzchowska-Renke, S. Ivancheva, and M. Kurteva, Effect of environment polluion on the composition of polyphenol and bioelements content in Achillea millefolium L. and Tanacetum vulgare L., Herba Pol. 1997; 43, 413–418

30. Oluwole AF, Asubiojo OI, Adekile AD, Filby RH, Bragg A and Grimm CI. Trace element distribution in the hair of sickle cell anemia patients and controls. J. Biol. Trac. Elm. Res. 1990; 26-27: 479-484
31. Central Council for Research in Ayurveda and Siddha. HPTLC-Fingerprint atlas of Ayurvedic single plant drugs mentioned in Ayurvedic Pharmacopoeia Vol-III and IV-A. report submitted to WHO, India, pp3-4
32. Shankar G, Garg KL. Nutritional value of some important fruits. Handbook of Horticulture, Kitabistan, Allahabad. 1967
33. Rathore M. Nutrient content of important fruit trees from arid zone of Rajasthan. J Hort Forestry 2009; 1:103-108.
34. Bhati, R., N.S. Shekhawat and H.C. Ary *In vitro* regeneration of plantlets from root segments of . Indian J. Exp. Biol. 1992; 30: 844-845.
35. Pati R and Muthukumar S. Histological and biochemical changes in *Aegle marmelos* Corr. Before and after acclimatization. Tree Genetics and Molecular Breeding 2013 3(3),12-18.
36. A.O.A.C. Official methods of analysis. Association of official analytical chemists. Inc. 17th ed. Arlington, Virginia. 2000. USA.
37. Dubois, M., Giller, K. A., Rebers, P. A. and Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956; 28: 350-356.
38. Lowry O. W., Rosebrough N. J., Farr A. C. and Randall R. J. "Protein measurements with folin-phenol reagent". J. Biol. Chem., 1951; 193: 255-257
39. The Ayurvedic Pharmacopoeia of India, Part I, volume-IV, Ministry of Health and Family Welfare, Department of Ayush, New Delhi, 1982.pp 13-14.
40. The Ayurvedic Pharmacopoeia of India, Part I, volume-III, Ministry of Health and Family Welfare, Department of Ayush, New Delhi, 1982,pp 29-31.
41. Vermani, A., Prabhat, N. and Chauhan, A. Physico-Chemical Analysis of Ash of Some Medicinal Plants Growing in Uttarakhand, India. Nature. Science.2010, 8(6): 88-91
42. Kelsay, J. L.Effects of diet fiber on bowel function and trace mineral balances of human subjects. Cereal Chem.1981; 58: 2-5.
43. Le Veille, G. and Sanberlich, H.E. Mechanism of the cholesterol- dressing effect of pectin in the cholesterol fed rat. J. Nutr.1966; 209-214.
44. Abolaji O. A., Adebayo A. H and Odesanmi, O. S. Nutritional Qualities of Three Medicinal Plant Parts (*Xylopia aethiopica, Blighia sapida and Parinari polyandra*) commonly used by Pregnant Women in the Western Part of Nigeria. Pak. J. Nut. 2007;6(6): 665-668
45. Narendhirakannan RT, Subramanian S, Kandaswamy M. "Mineral content of some medicinal plants used in the treatment of diabetes mellitus", Biol. Trace Elem. Res. 2005; 103(2): 109-115.
46. Singh U., Anita Kochhar A. and Boora R. Proximate Composition, available Carbohydrates, Dietary Fibres and Anti-Nutritional factors in BAEL (Aegle Maemelos L.) Leaf, Pulp and Seed Powder International Journal of Scientific and Research Publications 2012; Volume 2, Issue 4
47. Dandapat S. , Kumar A. and Sinha M.P. Antipathogenic efficacy of methanolic leaf extract of *Cinnamomum tamala* and with their nutritional potentiality. The Biocon; 2013 8 (2), Supplement on Medicinal Plants 635-641
48. Bhatti R., Singh J., Saxena A.K., Suri N. and Ishar M.P.S. Pharmacognostic standardisation and antiproliferative activity of (L.) Correa leaves in various human cancer cell lines. Ind J Pharm Sci. 2013 Nov-Dec; 75(6): 628-634.
49. Janarthanan U., Varadhrajan V. and Krishnamurthy V. Physicochemical Evaluation, Phytochemical Screening and Chromatographic Fingerprint profile of *Aegle marmelos* (L.) Leaf extracts. World Journal of Pharmaceutical Research; 2012, vol. 1(3); 813-837.
50. Khan SA, Ahmad I And Mohatir N.S. Evaluation of mineral contents of some edible medicinal plants. Pak J Pharm Sci 2006 Apr; 19(2): 148-52

51. Vinodhini R, Narayanan M. Cytoprotective effect of Nelumba nucifera and *Aegle marmelos* in common carp (Cyprinus carpio) exposed to heavy metals. International Journal of Integrative Biology 2009; 7(2): 124-129.
52. Annan K, Dickson A., Amponsah I and Nooni I. K. The Heavy metal contents of some selected medicinal plants sampled from different geographical location. Pharmacognosy Res. 2013 Apr-Jun; 5(2): 103–108.
53. Mihucz V.G, Tatar E., Kmethy B., Zaray G., and Cseh E., Investigation of transportedheavy metal ions in xylem sap of cucumber plants by size exclusion chromatographyand atomic absorption spectrometry, J. Inorg. Biochem. 2000 81, 81–87.
54. Heyliger C.E., Tahiliani A.G., and McNeill J.H., Effect of vanadate on elevated bloodglucose and depressed cardiac performance of diabetic rats, Science 1985; 227, 1474
55. Schecter Y. and Karlish S. J. D. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl(IV) ions, Nature 1980; 284, 556
56. Schecter Y and Ron A., Effects of depletion of phosphate and bicarbonate ions on insulin action in rat adipocytes, J. Biol. Chem. 1986; 261, 14,945
57. Rosetti L Giaccari A., Klein-Robbenharr A.E., and Vogel L.R., Insulinomimetic proper-ties of trace elements and characterization of their in vivo mode of action, Diabetes 1999, 39, 1243
58. V.G.Yuen, E.Vera, M.L.Battell, W. M. Li, and J. H. McNeil, Acute and chronic oral administration of bis (metallato) oxovanadium(IV) on fa/fa Zucker diabetic fatty (ZDF)rats, Diabetes Res. Clin. Pract. 1999, 43, 9
59. Scott D. A and Fisher A.M., The insulin and zinc content of normal and diabetic pancreas, J. Clin. Invest**.** 1938 17, 725–728 CrossRef
60. Levine A.S., McClain C.J, Handwerger B.S., Brown D.M., and Morley J.E., Tissue zinc status of genetically diabetic and streptozotocin induced diabetic mice, Am. J. Clin. Nutr. 1983 37, 382–386.
61. May J.M. Contreggi C.S., The mechanism of the insulin-like effects of ionic zinc, J. Biol. Chem.1982; 257**,** 4362–4368.
62. Mertz W., Chromium in human nutrition: a review, J. Nutr. 1993 123, 626–633.
63. William DM. Copper deficiency in humans, Semin Hematol, 1983, 20; 118-128
64. Anderson R.A., Chromium glucose tolerance, diabetes and lipid metabolism, J. Adv. Med. 1995 8**,** 37–49.
65. Anderon R.A., Recent advances in the clinical and biochemical manifestation of chromium deficiency in human and animal nutrition, J. Trace Elements Exp. Med. 1998 ,11, 241–250 CrossRef
66. Rajurkar N.S. and Damame M. M., Mineral content of medicinal plants used in the treatment of diseases resulting from urinary tract disorders, Appl. Radiat. Isot. 1998. 49**(**7**),** 773–776 CrossRef
67. Speck J.F.The effect of cations on the decarboxylation of oxaloacetic acid, J. Biol. Chem. 1949; 178, 315.
68. Kar A., Choudhary B.K.and Bandyopadyay N.K. Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test, J. Ethnopharmcol 1999. 64, 179–184.
69. Agarwal VS. Rural economics of medicinal plants: Vegetation in the forest. In: Drug plants of India. New Delhi, alyani Publishers, 1997, pp 1, 6, 44, 45, 102, 103, 129, 160.
70. Bael (*Aegle marmelos*). Medicinal plants, herbs, spices. The Tribune. Online edition. www.tribuneindia.com

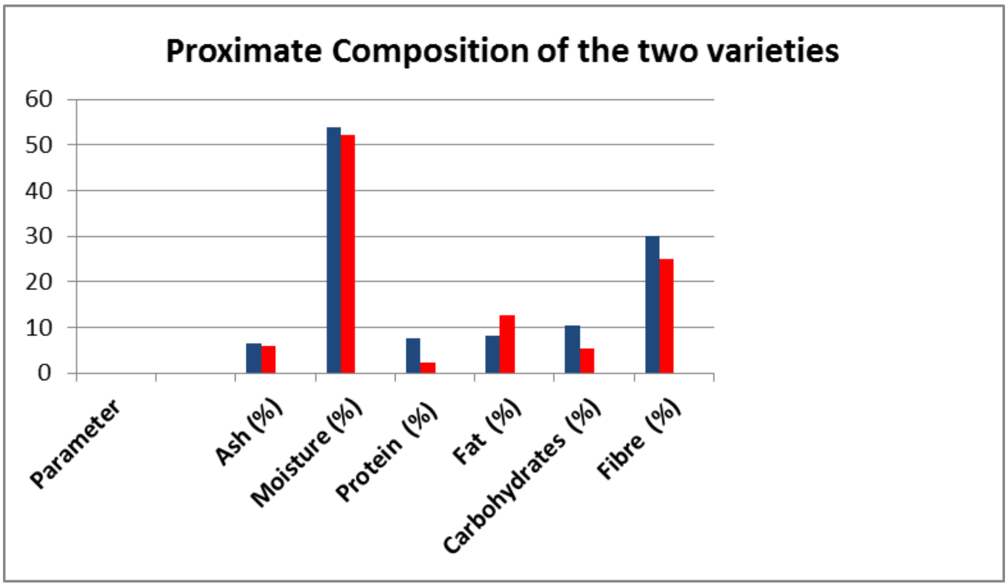

Fig 1: Proximate composition of the two varieties

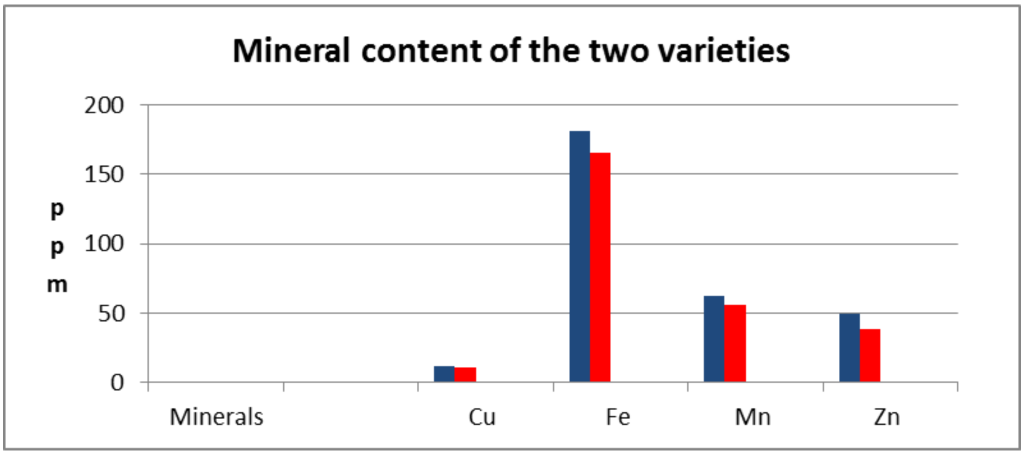

Fig 2: Comparison of the mineral content of the two varieties

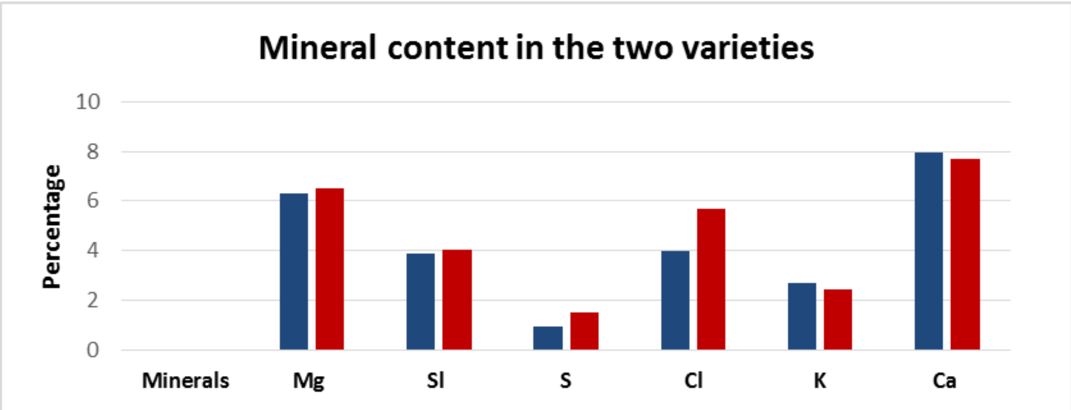

Fig 3: Trace elements in the two varieties

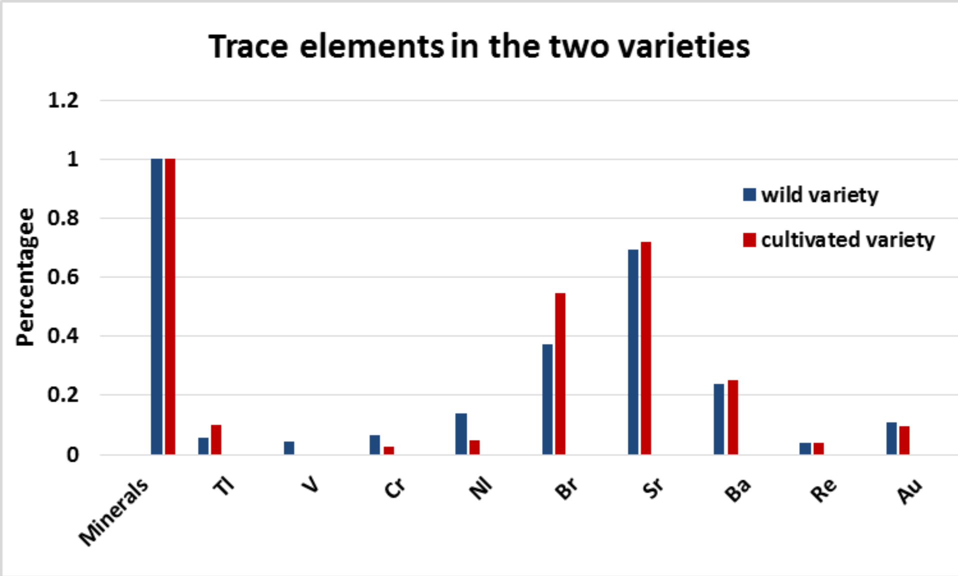

Fig 4: Trace elements in the two varieties