



Elemental and Phytochemicals Profile in Ethno Medicinal Plants Used for Treating Cardiovascular Diseases by Mizo-Tribe of North Eastern India

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Proton induced X-ray emission (PIXE) and gas chromatography-mass spectroscopy (GC-MS) were used to detect elements as well as phytochemicals in the *Centella asiatica* and *Elsholtzia communis* having possible roles for cardio-protections. Elements namely K, Cl, Ca, Fe, Mn, Zn and Cu were present in varying concentration with notable concentration of K, Cl and Fe. Phytochemicals such as stigmasterol, β -sitosterol, phytol and N-hexadeconic acid were detected in the *C. asiatica* while *E. communis* was found to contain β -carotene, β -sitosterol, phytol and N-hexadeconic acid. The study reveals that these two ethnomedicinal herbs are found to contain elements and phytochemical known for their cardio-protective roles.

Keywords: Bioactive compounds, Ethnomedicinal plants, Folklore medicines, Proton induced X-ray emission, GC-MS.

INTRODUCTION

In the past few decades, a widespread approach in medicinal field is the therapeutic usage of plant species in curing of various diseases due to presence of certain bioactive compounds, essential metallic elements, anti-inflammatory and antioxidant metabolites *etc.* A possible link to the therapeutic action of the medicinal plants is because of the reason that most of the medicinal plants are found to be rich in one or more individual elements and phytochemicals [1-3]. A number of elements essential to human nutrition are accumulated in the different part of plants as it accumulates minerals essential for growth from the environment [4]. On the other hand, bioactive compound or phytochemical is often used to describe large number of secondary metabolic compounds found in plants which play an important role in the competition, defence, attraction and signalling but are not necessary for the daily functioning of the plant such as growth [5]. These compounds are known to elicit pharmacological effects in humans and animals [6]. It has been reported that for treating of cardiovascular diseases and enhancing the immune system, body required small quantity of essential elements and phytochemicals

which defend from harmful reactive oxygen species (ROS) and other lipid peroxides [7,8]. Moreover, consumption of the plant derived phytochemical may drift the balance towards the adequate antioxidant status in the body as many medicinal plants contain large amounts of antioxidants, such as polyphenols, β -carotene, carotenoids, essential elements and plant secondary metabolites such as phytosterols, flavonoids and terpenoids which play an important role in defence against free radicals and other metabolic activities [7,9]. Due to deficiency of certain elements, oxidation of biomolecule is enhanced and produced more reactive oxygen species, which resulted damage of immune cells and dysfunction of mitochondrial cells, protein mediated membranes and several biomolecules which have been implicated in cardiovascular diseases such as atherosclerosis, hypertension and in endothelial dysfunction [10,11]. In this regard, metals coordinated antioxidant enzymes activities terminate the production of highly reactive oxygen species and acts as cardio protection while non-enzymatic antioxidants such as β -carotenoid derivative and other phytochemicals directly scavenge unpaired electrons of free radicals and thus prevent damage propagation of cardiovascular tissues [12,13]. In addition to this, the phytosterols in the medicinal

plants, use to their cholesterol lowering activities in the body are believed to contribute to beneficial effects for heart related diseases [14].

Mizoram is a remote State in North-Eastern region of India bordering Myanmar and Bangladesh and lies in the Indo-Burma biodiversity hotspots. The state has a rich diversity of ethnomedicinal plants and fruits and most of them are endemic to this region [15,16]. The tribal native of this region have a rich heritage about using plants in their folklore medicine in the treatment of various diseases like skin diseases, cancer, jaundice, diabetes and cardiovascular diseases *etc.* [17,18]. *Centella asiatica* (L.) Urb, (Family-Apiaceae) and *Elsholtzia communis* (Collett & Hemsl.) Diels, (Family-Lamiaceae) are herbaceous ethnomedicinal plants locally known as “Lamback” and “Lengser” respectively and widely used by traditional practitioner of this region in their folklore medicines for treating cardiovascular diseases [19,20]. Approximately on an average, 60 % total population of Mizoram having hypertension/or cardiovascular related diseases have used these forms of traditional medicine during the course of their disease management (unpublished data by JP Rajan from his field work). Besides their therapeutic usages, sometimes the leaves of *C. asiatica* is also eaten as vegetables while the dry inflorescence part of *E. communis* is used as spice in cooking popular delicious dishes due to their taste and flavour. The traditional practitioner of Mizo community in this region are claiming that low incidence of hypertensive people among their community member is also due to occasional intake of this medicinal herb as vegetables despite high consumption of meat based foods on regular basis. However, their therapeutic used is yet to be established.

Herein, the elements content and presence of phytochemical in these two medicinal plants were carried out by employing proton induced X-ray emission (PIXE) and gas chromatography-mass spectroscopy (GC-MS) techniques and their possible roles as cardio-protective agents is discussed for the first time. Proton induced X-ray emission is one way simultaneous, reliable, sensitive, quantitative multi-elemental and non-destructive suitable for routine analysis due to minimal sample preparation and less turn over time and it was earlier used for this purpose several times [17,21] while GC has a key technological platform for identification of plants phytochemical. The main emphasize of this study is on finding plant based bioactive compounds and elements with focus on the impactful way to translate tradition knowledge of the therapeutic usage of ethnomedicinal plants in the treatment of diseases that could facilitate meaningful scientific data and public health benefits in the context of prevention, delaying and curing of cardiovascular diseases. Thus, the objective of the present study is to determine essential elements and phytochemical profile in the two selected ethnomedicinal plants of the north-eastern India commonly used in their folklore medicines for treating cardiovascular diseases.

EXPERIMENTAL

C. asiatica arial parts and *E. communis* inflorescences were collected from the local market of Aizawl, Mizoram, India. The plant specimens were identified by Dr. H.S. Thapa, a taxonomist in the Department of Botany, Pachhunga

University College, a Constituent College of Mizoram University and was deposited at Department of Botany, Pachhunga University College, Aizawl as herbarium (Voucher specimen No 115 and 116 respectively). The plant materials of interest were shade dried for 3 weeks, ground into powder in an agate pestle mortar, passed through 60-mesh sieve and keep in an air tied plastic bottle for further analysis.

Preparation of samples for PIXE and GC-MS analysis:

For the PIXE analysis, 2 g of each powdered sample was mixed with high purity 0.2 g of graphite powder. The purpose of mixing graphite powder was to monitor the beam current. The mixture was pressed in to pellets of 2 mm thickness and 20 mm diameter with a pressure of 30 kPa. Pellets of certified reference materials bovine liver (NIST-1577b) was prepared in similar way for quantification and verification of the results.

In case of GC-MS analysis, 50 g of powder sample extracted by 60 % methanol by four times to obtained solution become colourless. The collected extract was filtered and the filtered liquor was concentrate using rotator evaporated to solvent remove and then vacuum drying in oven at 50 °C for 3 days. About 1 g of the solid residue of plant samples was dissolved again in methanol and injected 1 µL in GC-MS for identification.

Proton induced X-ray emission analysis for elements

content: The 3 MeV collimated proton beam, obtained from the 3 MV tandem pelletron accelerator at National Centre for Compositional Characterization of Materials (NCCCM), Department of Atomic Energy (DAE), Hyderabad, India, was used to irradiate. The scattering chamber consists of a multiple target holder with rotatable to manipulate the beam-target angle. The samples pellets were suspended on an aluminum plate then loaded in multiple target holder ladders and it was oriented at 45° to attained vertical position. The ladder was moved vertically inside PIXE chamber for maintain to same projectile target inside PIXE chamber under vacuum conditions (10^{-6} Torr). The irradiation was carried out with maximum beam current 10^{-8} Amperes, germanium detector as Eurisys Measures Type EGX 100-01, Be window thickness 40 µm, FWHM of 150 eV at 5.9 keV, mylar thickness 40 µm as an absorber and electron suppressor with -900 V in front of the sample was used. The spectral data were analyzed using GUPIX software package, which provides fits the element K α and K β peaks and converts raw spectral data into elemental concentration. The generated data were analyzed by GUPIX and ours results was checked against the certified values from the standard reference materials and was found to be good [17].

GC-MS analysis for identification of phytochemicals:

Phytochemical analysis of *C. asiatica* and *E. communis* by GC-MS was carried out using an Auto system XL gas chromatography (Perkin Elmer Instrument) system coupled to Turbo Mass Spectrophotometer (Perkin Elmer Instrument) installed at Sophisticated Instrumentation Centre for Applied Research and Testing, Vidyanagar, Gujarat, India. The PE-5MS fused silica capillary with 5 % diphenyl and 95 % dimethyl polysiloxane column (30 m \times 50 μ m \times 0.25 μ m). Helium gas was used as the carrier gas at a constant pressure 100 k pa with flow rate of 1.0 mL/min. The sample injection volume was 10 µL with (1:40) split mode and the column temperature was programmed set at range from 70-250 °C at the raised 10 °C/

min. The injector and detector temperatures were set at 250 and 280 °C, respectively. The ionization voltage was 70 eV with the mass scan operating in the range of 40-550 amu. The phytochemical identified by using comparison of retention time and mass fragmentation pattern obtained by turbo mass software of total ionic chromatogram (TIC) and search from NIST library with stored in computer library [22,23].

RESULTS AND DISCUSSION

Essential elements recorded in two medicinal plants:

The concentration of major and minor elements detected namely K, Cl, Ca, Fe, Mn, Zn and Cu after PIXE analysis in the *C. asiatica* and *E. communis* (Table-1) while PIXE spectra of both the plants are shown in Figs. 1 and 2, respectively. Analysis of elemental data reveals that K and Fe were generally observed with highest concentration in both the ethnomedicinal plants as compared to other major and minor elements recorded. The content of major elements recorded in the *C. asiatica* and *E. communis* were in the range of 28840 ± 961.5 mg/L to 2257 ± 612.8 mg/L, 5320 ± 215.4 mg/L to 494 ± 16.7 mg/L and 3431 ± 668.1 mg/L to 3209 ± 225.6 mg/L for K, Cl and Ca respectively with highest concentration of K, Cl and Ca recorded in *C. asiatica*. In the present study, trace elements were also recorded in varying concentration in both the medicinal plants studied. The content of the trace elements recorded were 1131.21 ± 123.4 mg/L and 651.18 ± 24.5 mg/L of Fe, 137.31 ± 16.4 mg/L and 30.76 ± 2.18 mg/L of Mn, 16.32 ± 2.5 mg/L and 28.03 ± 2.60 mg/L of Zn and 10.24 ± 2.4 mg/L and 8.92 ± 1.42 mg/L of Cu both in *C. asiatica* and *E. communis* respectively. The highest concentration of Fe, Mn and Cu were observed in *C. asiatica* while *E. communis* was found to be contained highest content of Zn. The results of the present study reveals that K was observed generally in higher concentration than the other major elements recorded with the highest concentration of K recorded in the *C. asiatica* (28840 ± 961.5 mg/L). K is the principal cation in intracellular fluid known for its vital roles for keeping healthy cardiac muscles like functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, proper cardiac muscle contraction, cell membrane function and Na⁺/K⁺-ATPase, otherwise may increased the chances of stroke [24,25]. Potassium also terminates the reactive oxygen species, hydroxide and nitric oxide free radical and reduces the formation of peroxide in plasma lipid in vascular tissue to help cardiovascular muscle work properly [26]. In addition to K, an appreciable amount of Cl and Ca were also recorded in both the ethnomedicinal plants with the highest concentration of Cl and Ca recorded in *C. asiatica* (5320 ± 215.4 mg/L and 3431 ± 668.1 mg/L respectively). Cl is an element making up 0.14 % of the concentration of elements in the human body known for its important roles of maintaining electrolyte function in the body. By combining with Na and K, Cl works well to keep all of our biological systems running smoothly and their imbalances in the body may leads to hypertension [27]. Likewise, Ca is an element which plays a pivotal in signal transduction pathways and low Ca intakes may be associated with cardiovascular diseases risk through pathways that affect blood pressure, insulin secretion as well as sensitivity and blood cholesterol concentrations

TABLE-1
MAJOR AND MINOR ELEMENTS CONTENT (mg/L)
IN TWO SELECTED ETHNOMEDICINAL PLANTS

Name of elements	<i>Centella asiatica</i> (Leave powder)	<i>Elostizia communis</i> (inflorescence powder)
K	28840 ± 961.5	2257 ± 612.8
Cl	5320 ± 215.4	494 ± 16.7
Ca	3431 ± 668.1	3209 ± 225.6
Fe	1131.21 ± 123.4	651.18 ± 24.5
Mn	137.31 ± 16.4	30.76 ± 2.18
Zn	16.32 ± 2.5	28.03 ± 2.60
Cu	10.24 ± 2.4	8.92 ± 1.52

Values are mean ± SD, 3 observations each.

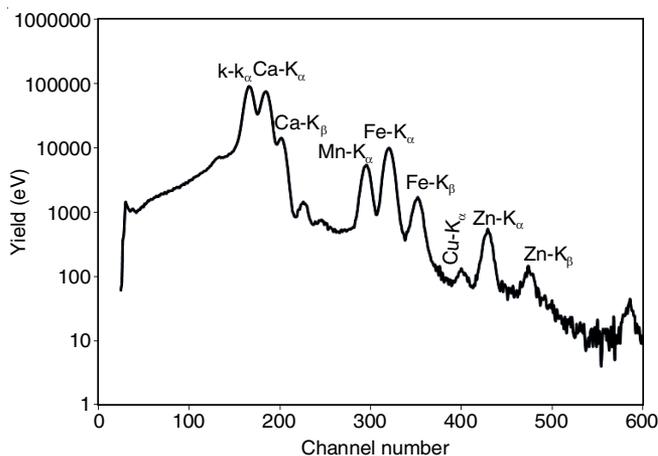


Fig. 1. Proton induced X-ray emission spectrum of *Centella asiatica*

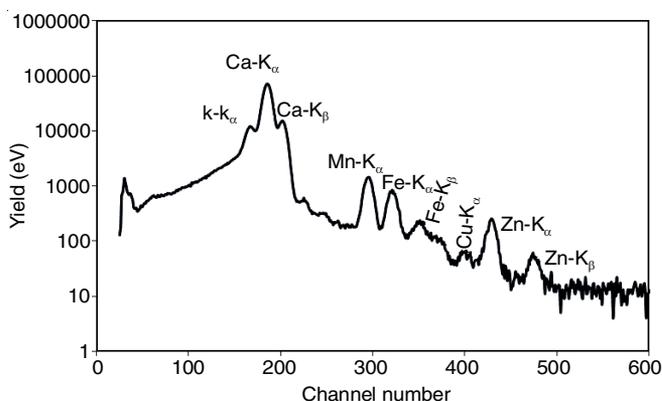


Fig. 2. Proton induced X-ray emission spectrum of *Elostizia communis*

[28,29]. In view of the above positive roles on cardiovascular related functions, the use of these two ethnomedicinal plants in the treatment of cardiovascular disease in the traditional practices may be attributed to considerable amounts of K, Cl and Ca present in them.

In the present study, Fe is generally observed higher than the other trace elements studied in both the ethnomedicinal plants with the highest concentration of Fe recorded in the *C. asiatica* (1131.21 ± 16.4 mg/L). It has been reported that adequate amount of Fe is required in the body to maintain healthy cardiac cells and proper functioning of the heart cells. During the progress of cardiovascular diseases, Fe is required for the growth of the RBCs as well as expansion of the blood volume which protected the cells from the oxidative injuries otherwise may lead to rapid damage of the cardiac cells [30].

In addition to Fe, the two medicinal plants were found to be contained notable amount of other essential trace elements like Mn, Zn and Cu with the highest concentration of Mn and Cu recorded in *C. asiatica* (137.31 ± 16.4 mg/L and 10.24 ± 2.4 mg/L respectively) while *E. communis* contained highest content of Zn (28.03 ± 2.60 mg/L). Manganese is an element required in the production of energy by regulating the metabolism of vitamin B₁, C, E, regulation of immune responses by proper catabolism of amino acids, by activation of enzymes important for proper digestion and utilization of foods [31]. In addition to this, Zn and Cu along with Mn act as an important component of the metallo protein structure of the enzymes of the antioxidant defense system like superoxide dismutase, catalase, cytochrome-c-oxidase, monochrome oxidase and dopamine monooxygenase which play important roles in scavenging reactive oxygen species during oxidative stress [32,33]. These trace elements are used for therapeutic purposes to prevent from the lipoprotein oxidation and free radical generation during oxidative damage in the heart and hence reduce damage of the heart cells [34,35]. Thus, the presence of the considerable amount of Fe, Mn, Zn and Cu in these two ethno-medicinal plants studied further support their use in treating cardiovascular diseases by the traditional practitioners of north-eastern India.

Phytochemical detected in the two medicinal plants:

Analysis of the GC-MS result indicates the presence of phytochemical like phytosterols, phytol, fatty acid and tetraterpene precursor in both ethnomedicinal plants studies. The GC-MS chromatogram of methanolic leaves extract of *C. asiatica* and methanolic inflorescence extract of *E. communis* are shown in Figs. 3 and 4 while the corresponding phytochemical profile are presented in Table-2, respectively. From both the GC-MS chromatogram, it was revealed that methanolic leaves extract of *E. communis* was found to contain phytochemical namely stigmasterol (C₂₉H₄₈O), β -sitosterol (C₂₉H₅₀O), phytol (C₂₀H₄₀O) and N-hexadeconic acid (C₁₆H₃₂O₂) while β -carotene (C₄₀H₅₆), β -sitosterol (C₂₉H₅₀O), phytol (C₂₀H₄₀O) and N-hexadeconic acid (C₁₆H₃₂O₂) were detected in the inflorescence extract of *E. communis*.

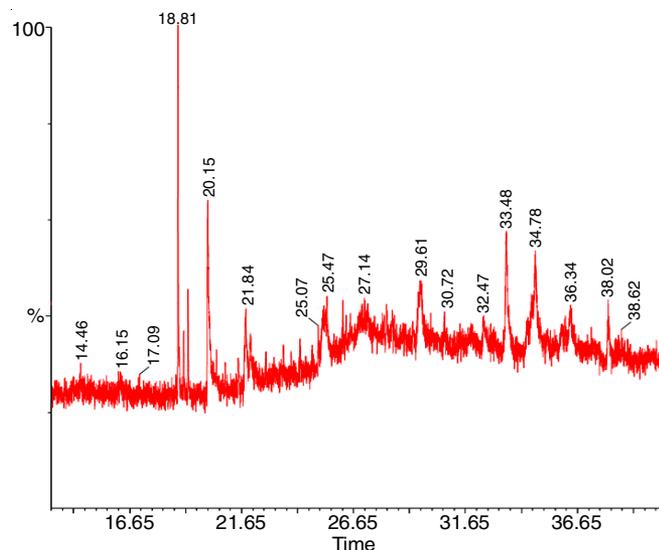


Fig. 3. GC-MS chromatogram of methanolic leaf extract of *Centella asiatica*

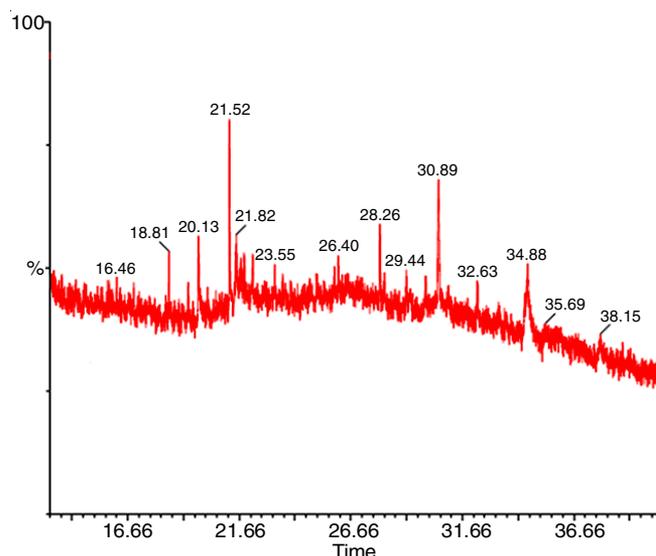


Fig. 4. GC-MS chromatogram of methanolic inflorescence extract of *Elostizia communis*

RT	Common name	m.w.	m.f.
33.48	Stigmasterol	412	C ₂₉ H ₄₈ O
34.78	β -Sitosterol	414	C ₂₉ H ₅₀ O
18.81	Phytol	296	C ₂₀ H ₄₀ O
20.15	N-hexadeconic acid	256	C ₁₆ H ₃₂ O ₂

Plant's phytochemical is known to elicit pharmacological effects in humans including reducing risk of and treatment for certain diseases. In the present study, methanolic leaf extract of *C. asiatica* was found to contain some of the known and common phytochemical namely stigmasterol (C₂₉H₄₈O), β -sitosterol (C₂₉H₅₀O), phytol (C₂₀H₄₀O) and N-hexadeconic acid (C₁₆H₃₂O₂), while β -carotene (C₄₀H₅₆), β -sitosterol (C₂₉H₅₀O), phytol (C₂₀H₄₀O) and N-hexadeconic acid (C₁₆H₃₂O₂), were detected in the inflorescence extract of *E. communis*. The phytosterols especially stigmasterol and β -sitosterol may play a pivotal role in maintaining healthy heart cells as these compounds are essential constituents of cellular membranes playing a key role stabilizing the phospholipids, which assist to maintain the fluidity and permeability of cell membrane of heart. Moreover, due to similarity in structure with cholesterol, these phytosterols may acts as cardio protective agents by their lipid lowering effects in the body as elevated level blood cholesterol is consider one major risk factor for the development of the cardiovascular diseases [36,37]. In this regards, McKenney *et al.* [38], also reported that the reductions of cholesterol in body can be achieved by consumption of esterified plant sterols. In intestine, phytosterols compete with absorption of cholesterol by binding to micelles, an important vehicle for cholesterol absorption and transport and thus reducing the cholesterol content of the lipid-laden micelles [14,39]. Moreover, it is believed that reactive oxygen species transfer in intracellular cardiac space as results of the damage of antioxidant enzymes leads to damage of the cardiovascular tissues and phytosterols may play a major role in reduction of free radicals in intra-

cellular space of by maintaining the integrity of phospholipids membranes of heart. Thus, the roles of phytosterols detected in prevention and treatment of cardiovascular and atherosclerotic diseases may be mediated not only by their cholesterol lowering properties but also through their anti-atherogenic activities [14,40]. In view of these beneficial roles, the possible use of these ethnomedicinal plants for treating cardiovascular diseases in the traditional practices can be understood due to presence of stigmasterol and β -sitosterol in these ethnomedicinal plants.

In addition to above, β -carotene may counteract pathogenesis of cardiovascular disease because of its ability to abate atherogenic processes by inhibition of peroxidation of cardiac-associated lipids and its free radical scavenging capacities [14,41]. In the present study, both the medicinal plants were found to present both phytol as well as linoleic acid namely N-hexadeconic acid known for its marked antioxidant and free radical scavenging potentials during oxidative stress in the cardiac cells. In this regards, it has been observed that synergistic antioxidant actions of these phytochemical protected albino rats from doxorubicin-induced myocardial necrosis [42]. In view of efficacy of these phytochemical as cardio-protective and anti-atherogenic agents, the use of these two ethnomedicinal plants in the folklore medicines for treating cardiovascular diseases is attributed to the presence of additional phytochemicals like β -carotene, phytol as well as N-hexadeconic acid in them.

Conclusion

In this study, PIXE technique was employed for the recording essential elements content while GC-MS techniques was used for the identification of phytochemical in these two medicinal plants. It is evident that the various essential elements and phytochemical present in these ethnomedicinal herbs have either direct/indirect or synergistic roles in the control and management of the cardiovascular related diseases. The results thus justify the usage of these two medicinal plants in the traditional practices for treating cardiovascular diseases since they are found to contain appreciable amount of essential elements like K, Cl, Ca, Fe, Mn, Zn and Cu and presence of phytochemicals like stigmasterol, β -sitosterol, β -carotene, phytol as well as N-hexadeconic acid in them. Outcomes of the present study may contribute to emerging insight into how traditional knowledge on widely available ethnomedicinal plant materials in the nature may be used to enhance strategies for maintenance of cardiovascular health and develop novel therapeutic approaches in order to find out unexplored efficacy for potential sources of chemically interesting and biologically important drug candidates and also better integration of traditional medicines into the national health system in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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