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MOLECULAR APPROACH TO DETERMINE THE HOT AND COLD TEMPERAMENTS IN PLANTS ACCORDING AVICENNA CONCEPTS, THE ROLE OF MAGNESIUM

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Background: The molecular approach of millenary notion about hot and cold temperaments (mizaj) in plants according to Avicenna concepts is not investigated. The aim of this study is to analyze the correlation between mizaj property of plants from the Tajikistan area and their amount/quality of metal.

Methods: We analyzed the presence of several metals that implicated in body's homeostasis integrity, such as sodium, potassium, calcium, and magnesium, in several edible plants (n=23) from different regions in Tajikistan. Samples (n=43) of plant's parts including seeds, flower, leaf, stem and root were analyzed for their elements' composition using Energy Dispersive X-Ray Analysis (EDX) which incorporates Scanning Electron Microscopy (SEM).

Results: We didn't find any correlation between the area of the collected samples and the amount of metal absorbance by the plants. The metals distributions of plant's parts varied from one to the other. In this study, we found strict correlation between the amount of magnesium and the temperament properties of the plants reported by Avicenna. The magnesium was detected in high amount in *Achillea millefolium* L., *Rosa canina* L., *Coriandrum sativum* L., *Foeniculum vulgare* Mill. and *Nigella sativa* L.

Conclusions: The presence of high concentration of magnesium in the edible plants can be considered as a metal contribution (factor) in hot mizaj property and may lead to energy regeneration and wellbeing after consumption.

Key words: *Metal, hot and cold nature, plants, Avicenna, magnesium, Tajikistan.*

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МОЛЕКУЛЯРНЫЙ ПОДХОД В ОПРЕДЕЛЕНИИ «ГОРЯЧЕЙ» И «ХОЛОДНОЙ» НАТУР РАСТЕНИЙ В СООТВЕТСТВИИ С КОНЦЕПЦИЯМИ АВИЦЕННЫ, РОЛЬ МАГНИЯ

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Молекулярный подход к концепции Авиценны о «горячем» и «холодном» мизаджах растений тысячелетней давности всё ещё не исследован.

Цель: анализ взаимосвязей между «мизаджами» растений, произрастающих на территории Таджикистана, и содержанием (количеством/качеством) металлов.

Материал и методы: проанализированы присутствие нескольких элементов, таких как натрий, калий, кальций и магний, которые принимают участие в процессах гомеостаза организма, в составе нескольких лекарственно-пищевых растений (n=23) из разных регионов Таджикистана. В образцах (n=43) отдельных частей растений (семена, цветки, листья, стебли и корни) был изучен состав элементов с использованием энергодисперсионного рентгеновского анализа на сканирующем электронном микроскопе.

Результаты: не обнаружено никакой корреляции между регионом сбора и количеством металлов, поглощённых растениями. Распределение металлов в различных частях растений варьировало. В этом исследовании обнаружена строгая корреляция между количеством магния и особенностями натуры растений, описанных Авиценной. Магний был обнаружен в большом количестве в *Achillea millefolium* L. (тысячелистник), *Rosa canina* L. (шиповник), *Coriandrum sativum* L. (кориандр), *Foeniculum vulgare* Mill. (фенхель) и *Nigella sativa* L. (чернушка).

Заключение: высокая концентрация магния в лекарственно-пищевых растениях может быть характерным свойством «горячего» мизаджа, и при их употреблении способствовать восстановлению энергетического потенциала и оздоровлению организма.

Ключевые слова: *металл, горячая и холодная натура, растение, Авиценна, магний, Таджикистан.*

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INTRODUCTION

According to Avicenna, the internal properties of the alimentations varied in temperaments and corresponded to both kinds of cold and hot properties. The balance of cold/hot was presented as a biological law in the Canon of medicine [1]. Disruption of this balance may stimulate metabolic syndrome [2]. There is little information about molecular as well as physiological basis of these concepts. We are interested in investigating the role of metals in some plants identified as hot or cold according to Avicenna.

Certain elements have long been recognized (known) to have important biological functions primarily as a consequence of nutritional investigations [3-5]. Thus, the absence of a specific, essential mineral from the diet of an organism invariably leads to a deficiency state characterized by metabolic abnormalities with altered body homeostasis. Because such metals are usually present in tissues in very small amounts, it was reasonable to suspect that they might play an important role as catalyst via participating in enzymatic reactions. The discovery of metallo-enzymes, however, required the availability of accurate, sensitive and analytical methodology. As a consequence, the unequivocal demonstration of a role for metals, in enzyme action, is of relatively recent vintage.

At present, reliable measurements of small concentrations of metals in tissues, cells, subcellular particles, body fluids and bio macromolecules can be performed among other methods by colorimetry, fluorimetry, polarography, emission spectrometry with spark, flame or plasma excitation sources, x-ray and atomic fluorescence, atomic absorption and neutron activation analysis. Metals that have been detected by such techniques and currently known to be components of metalloenzymes include manganese and zinc in various enzyme activity, cobalt (carboxylation), copper (oxido-reduction), iron (oxido-reduction), molybdenum (oxido-reduction), nickel (urease), selenium (peroxidase). Among these metals, over 300 enzymes require the presence of magnesium ions for their catalytic action [6], including all enzymes utilizing or synthesizing ATP [7] or those that use nucleotides to synthesize DNA and RNA [8, 9].

In central Asia, habitually, the medical properties of the plants or their fruits were also classed in the two temperaments (mizaj) cold and hot. Based on the Canon of Avicenna, several local investigations allowed identifying the medical possession of regional plants as well as their categories [10]. In recent study, we reported the presence of first main series of transition elements from atomic number of 22 to 29 such as titanium, vanadium, chromium, manganese, iron, cobalt, nickel and copper in the plants from Tajikistan areas [11] and observed that the majority of plants with hot mizaj property accumulate more metals when they compared with cold mizaj plants. Over all, the molecular approach of this milliner notion is not investigated.

The aim of this study is to analyze the correlation between mizaj property of plants and their amount of metal quality in medicinal plants.

Material and methods

Plants diversity and regions of collect

23 different plants according to their cold and hot properties were collected in several regions in Tajikistan. Their classification and distribution in Tajikistan area is presented in table.

Table *Hot* and cold** temperament plants reported by Avicenna****

№	Plants	Regions of collect	Date of collect	Altitude (m)
1	*Anethum graveolens* L.* (aboveground part)	Nourabad	July, 2017	800-1800
	Anethum graveolens L. (seeds)	Faizabad	August – September, 2016	1500-1630
2	*Thymus seravshanicus* Klok.*	Nourabad	July, 2017	1700-3600
3	*Achillea millefolium* L.*	Nourabad	July, 2017	1800-2400
4	*Tanacetum pseudoachillea* C. Winkl.*	Nourabad	July, 2017	1600-3000
5	*Peganum harmala* L.*	Nourabad	July, 2017	450-3700
6	*Origanum tyttanthum* Gontsch.*	Nourabad	July, 2017	800-2700
7	*Hypericum perforatum* L.*	Nourabad	July, 2017	700-3000
8	*Ribes meyeri* Maxim.*	Rasht	2016	2000-4300
9	*Matricaria recutita* L.*	Ramit	June, 2017	400-1500
10	*Mentha arvensis* L.*	Ramit	June, 2017	850-2000
11	*Prunus armeniaca* L.**	Gonchi	June, 2017	800-2480
12	*Prunus cerasifera* Ehrh.**	Gonchi	June, 2017	400-2600
13	*Berberis nummularia* Bunge.**	Condara	2016	1500-2500
14	*Rhus coriaria* L.**	Ramit	2016	1000-1800
15	*Rheum maximoviczii* Losinsk. **	Varzob	2016	1100-3900
16	*Rosa canina* L*.	Varzob	2016	1000-2200
17	*Glycyrrhiza glabra* L.*	Shahritous	2016	350-1600
18	*Coriandrum sativum* L. *	Faizabad	2016	400-1200
19	*Plantago lanceolata* L.**	Ramit	2016	600-3400
20	*Nigella sativa* L.*	Zafarabad	2016	1200-2000
21	*Foeniculum vulgare* Mill.*	Varzob	2016	900-2000
22	*Helichrysum thianschanicum* Regel.*	Varzob	2016	1800-3100
23	*Citrus limon* (*Citrus* Meyeri Yu.Tanaka)**	Dushanbe	2017	400-1200

***Avicenna «Works». Volume 13. Canon of medical science: The second book. Dushanbe: Donish; 2012. 958 p. Note: The numbers 8, 15 and 22 are not noted in the Avicenna's works. In the same book of Avicenna page 418-420, *Coriandrum sativum* L. was classed among of cold temperament plants, and explain that Claudius Galenus considered this plant as hot temperament.

Plant collection and extraction

All collected plants' parts such as flower, leaf, stem and root were collected and delicately washed by abundant distilled water and were dried in the dark for five weeks. The dried plant parts were grounded using a blender apparatus. The powders were kept in a special dish and sent for extraction to the University of Sorbonne Paris Cité-Paris 7, Lariboisière Hospital, INSERM U965, 75010 Paris, France.

Plant extraction was performed using 10 gr. of plant powder in 100 ml ethanol solution 70% diluted in distilled water and incubated in obscurity condition for 3 weeks under mixture apparatus (agitation). The plant powder solutions were centrifuged (5000 T/min for 30 minutes) and the supernatants were collected. The soluble plant extracts were dried and used for metal analysis.

Metal analysis

Multi-walls slide (Fig. 1a) was used to deposit 10 to 20µl of each sample. Slide then left to dry and prepared for analysis. Slide was coated by carbon layer to avoid the overcharge of the image (Fig. 1b) then EDX spectrum (Zeiss Ultra 55 FEG SEM with GEMIN, S260 CAMBRIDGE scanning electron microscopy) was used (Fig. 1c) to read the continent of the sample. For each sample, two to three zones were measured. The final of 2 to 3 data was expressed as the atomic and mass percent.

Energy Dispersive X-Ray Analysis (EDX) is an x-ray technique used to identify the elemental composition of materials. Applications include materials and product research, troubleshooting, deformulation, and more. For this study, EDX system was attached to Electron Microscopy instruments (Scanning Electron Microscopy (SEM). Energy Dispersive X-ray Spectroscopy is based on the detection of characteristic x-rays emitted of an element as a result of the de-excitation of core electron holes created by a high energy electron beam. An electron from a higher binding energy electron level falls into the core hole and an x-ray with the energy of the difference of the electron level binding energies is emitted. Due to the quantization of electron energy levels, the emitted characteristic x-ray energies for elements will generally be different from one element to another with only a few spectral peaks overlapping. The normal electron beam of a scanning electron microscope is used as the excitation source. The addition of an energy measuring x-ray detector with a thin, low mass element window on a SEM enables EDX for all elements from carbon on up in atomic mass. A spectrum consists of all x-rays characteristic emitted by the present elements in the sample on a continuous background due to Bremsstrahlung x-ray emission caused by the deceleration of the high energy electrons of the electron beam in the sample.

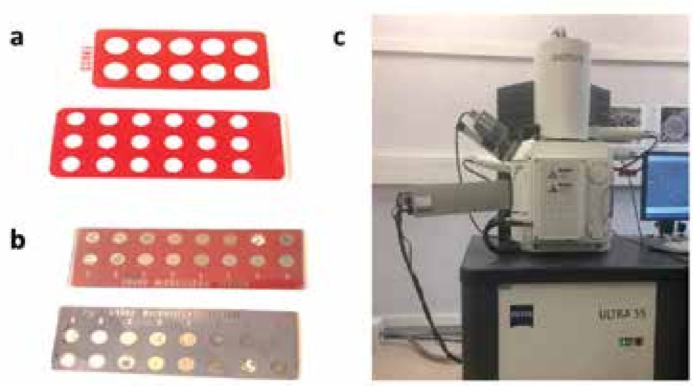
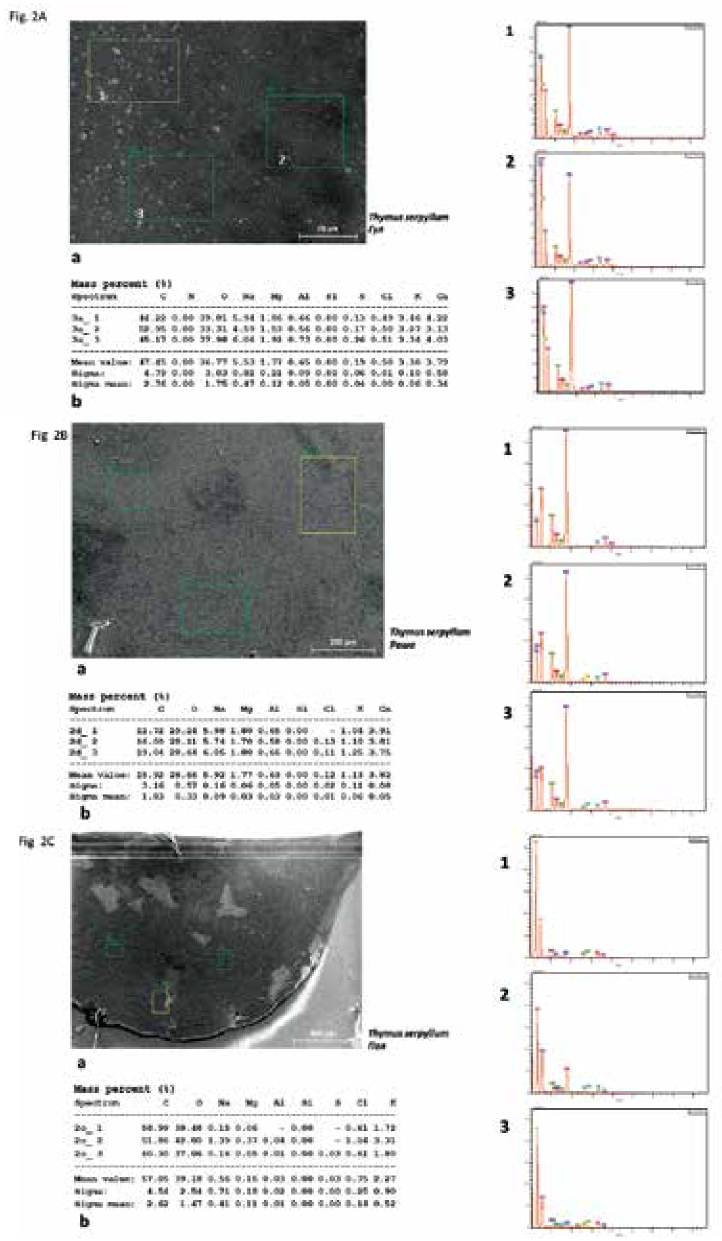


Fig. 1 *Samples preparation for scanning electronical microscopy SEM: multi-wall slide form (a); carbon layer coating the samples (b); scanning electronical microscopy machine (c).*

Results and discussion

Thymus seravshanicus Klok. was an illustrative example from all studied plants presented for flowers (Fig. 2a), roots (Fig. 2b) and stems (Fig. 2c). The three-wire specimen shown (a) was created to illustrate specific capabilities of the combined SEM/EDX system. This includes SEM imaging by secondary electrons and backscattered electrons

Fig. 2 *Thymus seravshanicus Klok. The highlighted rectangles in the inset image show the selected EDX inspection field (a). The detected spectrum and tabulated results revealed that Na, Mg, Al, K, and Ca are the main elements present with a variation of Mg and K amounts in each part of Thymus seravshanicus Klok. In this sample C, O, S, and Cl also presented. The relative amount of each detected metal is presented in a table in corresponding to the samples. In this study 15 KeV (in X axe) was used for detection of mineral composition in each simple and the % of minerals for different atom in each rectangle were expressed.*



in combination with compositional analysis and elemental mapping using EDX. Three parts of each sample were presented here and in each part, the semi-quantitative elemental composition information was performed for several metals that were found in 2-3×1000 nm depth. X-rays that have sufficient energy to escape the material surface can be detected as resulting in a spectrum of peaks with characteristic energies. The elements present in each zone showed in (1-3) and the amount of detected elements are presented in Fig. 2b. Na, Mg, Al, K, and Ca are the main metals and also C, O, Si and Cl elements were found. In this study, we focused on the presence of magnesium in the plants.

The amount of the metals Mg^{2+} detected for 43 parts from 23 plants collected from different regions in Tajikistan presented in Fig. 3.

The findings indicated that the amount of metals are varied according to plant's parts. In our example (*Thymus seravshanicus* Klok.), the analysis showed that the amount of the elements was not the same in roots compared to the flowers and stems. The first five plants with high amount of magnesium such as *Achillea millefolium* L., *Rosa canina* L., *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Nigella sativa* L. exhibited low amount of potassium and vice-versa for magnesium.

In this study, we found strict correlation between the amount of magnesium and temperament properties of the plants. In parallel, in other study, we analyzed the presence of copper and zinc and we did not find any correlation between amount of these metals and temperament properties (results not shown).

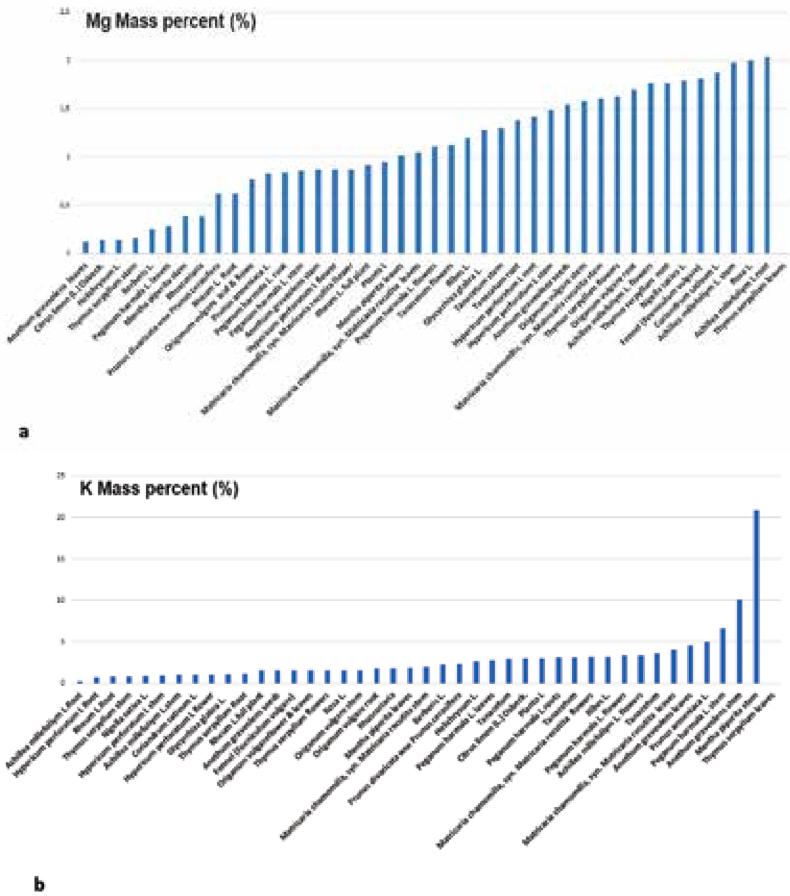


Fig. 3 *The relative distribution of magnesium (a) and potassium (b) in different plant's parts (mass percent).*

Mg^{2+} is the fourth-most-abundant metal ion in cells and the most abundant free divalent cation. As a result, it is deeply and intrinsically woven into cellular metabolism. Indeed, Mg^{2+}-dependent enzymes appears in virtually every metabolic pathway: specific binding of Mg^{2+} to biological membranes is frequently observed. Mg^{2+} is also used as a signaling molecule, and much of nucleic acid biochemistry requires Mg^{2+}, including all reactions that require release of energy from ATP [12-14], which is called ATP and is often actually Mg-ATP. In nucleotides, the triple-phosphate moiety of the compound is invariably stabilized by association with Mg^{2+} in all enzymatic processes [15].

Our results demonstrated that magnesium can be a candidate for hot temperaments of plants used currently as edible vegetable. The human body contains around 25 gr. of magnesium, 50 to 60 percent of which is stored in the skeletal system. The rest is present in muscle, soft tissues, and bodily fluids. Magnesium plays a role in over 300 enzymatic reactions within the body [16-21], including the metabolism of food, synthesis of fatty acids and proteins, and the transmission of nerve impulses. Magnesium is important for bone formation. It helps assimilate calcium into the bone and plays a role in activating vitamin D in the kidneys. Vitamin D is also essential for healthy bones [22]. Optimal magnesium intake is associated with greater bone density, improved bone crystal formation, and a lower risk of osteoporosis in women after menopause. Without magnesium, a high intake of calcium can increase the risk of arterial calcification and cardiovascular disease, as well as kidney stones. Thus, taking calcium supplements should also take magnesium to ensure their calcium intake is properly metabolized.

Magnesium plays an important role in carbohydrate and glucose metabolism, so magnesium status can also impact the risk of diabetes [23]. Several studies have associated a higher intake of magnesium with a lower risk of diabetes. For every 100 mg per day increase in magnesium intake, up to a point, the risk of developing type 2 diabetes decreases by approximately 15 percent. Low magnesium levels were linked to impaired insulin secretion and lower insulin sensitivity. According to the National Institutes of Health (NIH), the American Diabetes Association notes that further evidence is needed before magnesium can be routinely used for glycemic control in patients with diabetes. In previous study, we reported the influence of cold temperament diet on metabolic disorders and diabetes induction in animal models [24]. In parallel, magnesium is necessary to maintain the health of muscles, including the heart, and for the transmission of electrical signals in the body. These observations suggest the crucial role of magnesium in human body homeostasis especially in energy production processes. Presence of high concentration of magnesium in the edible plants with hot mizaj properties may contribute to energy regeneration and wellbeing after their consumption.

Deficiency of magnesium is linked with the symptom as numbness and tingling, muscle cramps, seizures, personality changes, heart rhythm changes and spasms. Deficiency also is associated with insulin resistance, metabolic syndrome, coronary heart disease, and osteoporosis. It can lead to low calcium or low potassium levels in the blood. The use of the plants with hot mizaj property can be interesting for treated magnesium deficiency as a traditional medication.

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