Journal of Pharmaceutical Research International



33(16): 76-85, 2021; Article no.JPRI.66181 ISSN: 2456-9119 (Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Doses of Ca²⁺, Mg²⁺, K⁺, Na⁺, Mn²⁺ and Zn²⁺ in Mountain Spring and Mineral Waters in Hydration Reaction During Sport Training and Recovery; Combination with Vitamins

Ignat Ignatov^{1*}, Mariana Angelcheva² and Ivan Angushev³

¹Scientific Research Center of Medical Biophysics (SRCMB), Sofia, Bulgaria. ²Department of Kinesitherapy and Rehabilitation, National Sports Academy "V. Levski", Bulgaria. ³Medical University of Sofia, Sofia, Bulgaria.

Authors' contributions

This work was carried out in collaboration among all authors. Author II designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MA and IA managed the analyses of the study. Author II managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i1631299 <u>Editor(s):</u> (1) Dr. Jongwha Chang, University of Texas, USA. <u>Reviewers:</u> (1) Janeth Marlene Quispe Avilés, University of São Paulo-USP, Brazil. (2) Nabeel Abdulkadhim Athab AL-Mashaakhi, University of Babylon, Iraq. Complete Peer review History: <u>http://www.sdiarticle4.com/review-history/66181</u>

Review Article

Received 14 January 2021 Accepted 18 March 2021 Published 26 March 2021

ABSTRACT

The human body consists of 55-60% water in the case of young adults. There are numerous studies conducted in the 21st c. concerning hydration reaction in sports training and recovery. Dehydration is a natural process during training and competition. Athletes lose both water and minerals. This is accompanied by formation of lactic acid and inorganic phosphate as well as by decrease of calcium (Ca²⁺). We have studied optimal doses of water and mineral intake during training, post-competition recovery and rest days. Electrochemically activated *Catholyte* water was successfully applied in sports practice. Bulgarian mineral and mountain spring waters were proposed as favorable during athletes' training. In addition, herbs used as food supplements were classified according to their vitamin content. Our aim was to verify the efficiency of proposed intake doses for athletes' hydration. They also include vitamins which, in combination with water, have anti-inflammatory effect and enhance sports performance. We have observed a tendency towards faster elimination of biochemical compounds appearing as side effects of athletic competitions and training.

Keywords: Hydration; sport; mountain and mineral waters; catholyte water.

1. INTRODUCTION

For high achievements at competitions, athletes should be properly hydrated. More than 2% of their body mass loss indicate significant dehydration. For an 80 kg athlete this is equal to 1.6 kg. Recommended daily water intake for healthy adults is 30 ml/kg. Thus, an 80 kg athlete should drink 2.4 I per day. Moreover, it has been shown that, after training and competitions, adequate daily water intake should be 40 ml/kg [1,2]. In the same example of 80 kg body weight, it is equal to 3.2 I per day. That is why; all doses calculated in our work are based on 3.0 I per day.

During a 90-minute football match, physical loading is related to endurance and speed. Dehydration of the human body reduces blood flow to the skin and increases hyperthermia [3]. In turn, heat dissipation is reduced. Due to hyperthermia and dehydration, heart pumping ability decreases. Consequently, blood flow to the muscles and their endurance are diminished. The body draws energy from Adenosine triphosphate (ATP). Chemical energy, produced by mitochondria, is stored in small adenosine triphosphate (ATP) molecules. ATP hydrolysis is described by the equation:

enzyme ATP+ $H_2O \rightarrow ADP+P+H^+$

Fatigue is caused by metabolic by-products. Current studies show that muscle fatigue is due to the lactic acid increase and concentration of inorganic phosphate (Pi) and hydrogen ions (H^+). There is a decrease of calcium (Ca²⁺) ions and glucose (Glc) concentration [4,5]. Hydrogen has reduction and oxidation properties and estimation of the effects depends on each case with intake of minerals and vitamins. The estimation is possible of study of saliva [6].

The aim of this paper, related to football training, is to show the dependence of hydration degree on water quantity and quality. Adequate amounts of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), manganese (Mg^{2+}) and zinc (Zn^{2+}) in mountain spring and mineral waters intake during sports training and recovery were determined. Combinations with vitamins, used in sports training and recovery, were also studied.

Vitamins were administered as food supplements extracted from Bulgarian plants and herbs. Effects of Catholyte water on athletes' recovery were also observed. Influence of Catholyte water on ATP synthesis and restoration of alkaline-acid balance was detected.

2. METHODS

2.1 Physicochemical Measurements of Mountain Spring and Mineral Waters from Bulgaria

Comparative analysis of physicochemical composition of mountain spring and mineral waters was performed.

2.2 Catholyte and Anolyte Activated Water Preparation [7-10]

Catholyte and anolyte activated water fractions were prepared by water electrolysis. The working principle of the electrolyzer is presented in Fig. 1.

The main stage of the electrochemical treatment of water takes place in an electrolyzer consisting of a cathode and an anode separated by a special semipermeable membrane (diaphragm). The diaphragm separates water into an alkaline fraction (catholyte) and an acidic one (anolyte). When electric current passes through water, the flow of electrons from the cathode as well as extraction of electrons from water at the anodeare accompanied by a series of redox reactions on the surfaces of both electrodes.

2.3 Comparative Analysis of Hydration, Dehydration and Heart Rate

Heart rate of football players was measured with GPS Sports Watches.

The results were statistically analyzed with Student's t-test.

2.4 Regulatory Compliance of Drinking Water Characteristics

All variants of drinking water consumed by research participants complied with the Bulgarian standards listed in Ordinance No 9 / 2001, Official State Gazette, issue 30, and decree No 178 / 23.07.2004.

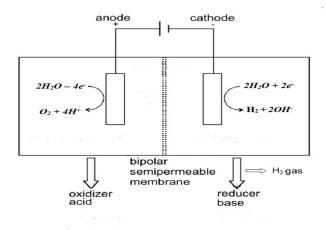


Fig. 1. Working principle of the electrolyzer

2.4.1 Methods for physicochemical analysis

- Method for determination of color according to Rublyovska Scale – method by Bulgarian State Standard (BDS) 8451: 1977;
- Method for determination of smell at 20°C

 method BDS 8451: 1977 technical device glass mercury thermometer, conditions No 21;
- Method for determination of turbidity EN ISO 7027, technical device turbidimeter type TURB 355 IR ID No 200807088;
- Method for determination of pH BDS 3424: 1981, technical device pH meter type UB10 ID NoUB10128148;
- Method for determination of oxidisability BDS 3413: 1981;
- Method for determination of chlorides BDS 3414: 1980;
- Method for determination of nitrates Validated Laboratory Method (VLM) – NO3 – No 2, technical device photometer "NOVA 60 A" ID No 08450505:
- Method for determination of nitrites VLM NO₂ – No 3, technical device photometer "NOVA 60 A" ID No 08450505;
- Method for determination of ammonium ions – VLM – NO₄ – No 1, technical device photometer "NOVA 60 A" ID No 08450505;
- 10. Method for determination of general hardness BDS ISO 6058;
- Method for determination of sulphates VLM - SO4 – No 4, technical device photometer "NOVA 60 A" ID No 08450505;
- Method for determination of calcium BDS ISO 6058;
- Method for determination of magnesium BDS 7211: 1982;

- Method for determination of phosphates VLM - PO4 – No 5, technical device photometer "NOVA 60 A" ID No 08450505;
- Method for determination of manganese VLM – Mn – No 7, technical device photometer "NOVA 60 A" ID № 08450505;
- Method for determination of iron VLM Fe – No 6, technical device photometer "NOVA 60 A" ID No 08450505;
- Method for determination of fluorides VLM – F – No 8, technical device photometer "NOVA 60 A" ID No 08450505;
- Method for determination of electrical conductivity – BDS EN 27888, technical device – conductivity meter inoLabcond 720 ID No 11081137.

The following characteristics, according to Ordinance No9, of investigated drinking water for sports purposes, are presented in Table 1.

3. RESULTS AND DISCUSSION

Field work results from tests performed by Ivaylo Yakimov in 2014 demonstrated the significance of hydration in sports training [11,12]. Possibilities to enhance hydration by improving water quality and precision of daily intake determination have been considered [13]. In sports training, analytical methods have been applied especially since the beginning of the 21st century. New opportunities for fitness and recovery are being sought [14,15]. Paik et al. consider reduction of oxidative stress during post-training hydration [16].

In our opinion, at this particular stage of sports training, it is necessary to use mineral and spring waters in certain proportions for most efficient hydration reaction. Combined intake of mountain spring and mineral waters could extensively be applied to improve this process for Bulgarian athletes during training, competitions and recovery. In addition, combination of such waters with electrochemically activated Catholyte water, especially in the recovery stage, improves functional capacity of the heart, activates bioprocesses in ATP synthesis and mitochondrial functions. Good hydration also helps reducing travel fatigue in modern sports.

3.1 Hydration Influence on Heart Rate

In 2019, Ignatov performed hydration tests on 10 football players in Nagano, Japan [6]. Their recovery heart rate was continuously measured from interruption of physical loading to the moment it reached the level of 130 beats per minute (bpm). All research participants reached the recovery heart rate target significantly faster when hydrated. In addition, the result with Catholyte water was better than that with table water (Table 2 and Fig. 2).

Comparison between the three columns noted as DH, H with table water, and H with Catholyte water was performed with Student's t-test. Average values m and standard deviationss are

estimated with t-criteria of Student. There is significant difference (P<0.05) between the first column and the third one. However, there is no significant difference between the first and second column, and between the second and third column.

This result shows that most efficient hydration is possible with Catholyte water. We also propose combinations of spring and mineral waters with Catholyte water.

Bulgaria has a great variety of mountain spring and mineral waters. For the purpose of this study, we selected for athletes' hydration several brands of bottled waters available on the market.

3.2 Waters Rich in Sodium (Na⁺) and Calcium (Ca²⁺) lons Bottled by Bulgarian Companies

3.2.1 Sodium (Na) rich water

Tables 3 to 6 show sodium-rich waters bottled by some Bulgarian companies.

3.2.2 Waters rich in calcium (Ca²⁺)

Table 7 shows calcium-rich water bottled by Bulgarian companies.

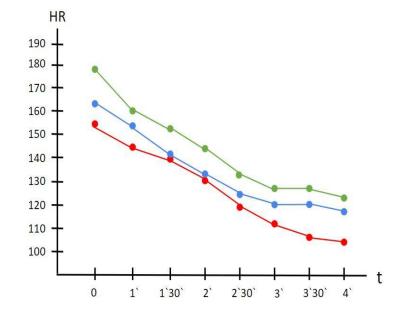


Fig. 2. Heart rate from interruption of physical loading to the moment it reached the level of 130 beats per minute (bpm), H (hydration) – DH (dehydration), DH (green color); H with table water (blue color), H with Catholyte water (red color), HR recov – bpm

Controlled characteristics of ions	Maximum limit value (mg/dm ³)
Sodium (Na)	200
Potassium (K)	-
Calcium (Ca)	150
Magnesium (Mg)	80
Manganese (Mn) µg/dm ³	50
Zinc (Zn)	4.0
Other characteristics	
рН	≥ 6.5 and ≤ 9.5

Table 1. Characteristics of investigated drinking waters for sports purposes according to Ordinance No. 9

Table 2. Heart rate from interruption of physical loading to the moment it reached the level of 130 beats per minute (bpm), H (hydration) – DH (dehydration), HR recovery – bpm

Parameters	DH	H with table water	H with Catholyte water
Interruption	179.3	163.3	154.1
1`	161.2	152.5	143.0
1` 30``	153.5	142.3	140.3
2`	144.0	135.7	132.3
2`30``	133.4	126.1	118.2
3`	131.5	122.0	113.4
3` 30``	130.2	121.5	106.5
4`	128.3	118.5	105.3

Table 3. Gorna Bania Mineral Water

Controlled parameters of ions	Result and Measuring unit(mg /dm³)	Maximum limit value (mg /dm ³)
Sodium(Na)	28.0	200
Potassium(K)	0.4	-
Calcium(Ca)	1.6	150
Magnesium(Mg)		80
Manganese(Mn)		50
Zinc(Zn)	<0.081	4.0
Other parameters	Result	
рН	9.80	≥ 6,5 and ≤ 9,5

Table 4. Bankia Mineral Water

Controlled parameters of ions	Result and Measuring unit (mg /dm³)	Maximum limit value (mg /dm ³)
Sodium(Na)	84.2	200
Potassium(K)	0.6	-
Calcium(Ca)	8.3	150
Magnesium(Mg)	-	80
Manganese(Mn)	-	50
Zinc(Zn)	-	4.0
Other parameters	Result	
рН	8.71	≥ 6,5 and ≤ 9,5

Controlled parameters of ions	Result and Measuring unit (mg /dm ³)	Maximum limit value (mg /dm ³)
Sodium (Na)	37.4	200
Potassium (K)	0.5	-
Calcium (Ca)	2.0	150
Magnesium (Mg)	-	80
Manganese (Mn)	-	50
Zinc (Zn)	-	4.0
Other parameters	Result	
pH	9.28	≥ 6,5 и ≤ 9,5

Table 5. Velingrad Mineral Water

Table 6. Hisar Mineral Water

Controlled parameters of ions	Result and Measuring unit (mg /dm³)	Maximum limit value (mg /dm ³)
Sodium (Na)	50.3	200
Potassium (K)	1.6	-
Calcium (Ca)	3.1	150
Magnesium (Mg)	-	80
Manganese (Mn)	-	50
Zinc (Zn)	-	4.0
Other parameters	Result	
pH	8.98	≥ 6,5 and ≤ 9,5

Table 7. Bachkovo Spring Water

Controlled parameters of ions	Result and Measuring unit (mg /dm³)	Maximum limit value (mg /dm ³)
Sodium (Na)	20.0	200
Potassium (K)	3.1	-
Calcium (Ca)	70.7	150
Magnesium (Mg)	7.8	80
Manganese (Mn)	-	50
Zinc (Zn)	-	4.0
Other parameters	Result	
pH	7.23	≥ 6,5 and ≤ 9,5

3.2.3 Waters containing almost equal amounts of sodium (Na⁺) and calcium (Ca²⁺)

Tables 8 and 9 show two brands of waters containing almost equal amounts of sodium (Na⁺) and calcium (Na⁺), bottled by different Bulgarian companies.

3.3 Waters Rich In Potassium (K⁺)

Analysis has shown that *Bachkovo* mineral water is rich in potassium (K^+). In Bulgaria there are sports complexes near mountain and mineral spring waters. In the town of Teteven, there is a sports complex where a mountain spring was built in1934. Its water composition is suitable for sports training (Table 10).

3.4 Suggested Mountain Spring And Mineral Waters For Drinking In Sports Training And Recovery

Analyses by Ignatov, Mosin et al. of Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Mn^{2+} and Zn^{2+} content in the human body for obtaining optimal balance of enzymes and vitamins was performed by studying blood indicators of healthy and cancer patients [17-20]. There are valid the following standards for the mountain spring and mineral waters in Bulgaria [21-23]

It showed that combinations of different mountain spring and mineral waterscan be applied in certain proportions for drinking during sports training and recovery.

3.4.1 Application in sports training

Recommended intake of *Velingrad, Bankya* or *Hisar* mineral water is 2 l/day, combined with 1 l/day of *Bachkovo* mineral water. Table 11 shows the corresponding mineral contents of such combinations.

3.4.2 Application in sports recovery

Catholyte water only is recommended for drinking during the first two hours of sports recovery. Then, *Bachkovo* and *Gorna Banya* mineral waters should be consumed.

Statistical analysis shows that most efficient hydration is achieved with Catholyte water.

Different brands of table water do not show statistically significant differences in that respect. That is why, we propose a hydration modality based on measurements of players after football matches. *Gorna Banya* water is suggested for its high pH=9.8. Excessive negative charges in Catholyte and in waters with high pH values contribute to neutralization of oxidative processes. In addition, *Bachkovo* mineral water efficiently provides calcium at 70.7 mg /dm³.

3.4.3 Application during rest days

Devin mineral water (1 I) and *Rilana* spring (1 I) water are recommending for drinking during rest days. Table 12 shows the mineral contents of such a combined intake.

Table 8. Devin Mineral Water

Controlled parameters of ions	Result and Measuring unit (mg /dm³)	Maximum limit value
Sodium (Na)	5.7	200
Potassium (K)	-	-
Calcium (Ca)	6.1	150
Magnesium (Mg)	0.3	80
Manganese (Mn)	-	50
Zinc (Zn)	-	4.0
Other parameters	Result	
рН	8.98	≥ 6,5 and ≤ 9,5

Table 9. Rilana Spring Water

Controlled parameters of ions	Result and Measuring unit (mg /dm³)	Maximum limit value (mg /dm³)
Sodium (Na)	4.1	200
Potassium (K)	-	-
Calcium (Ca)	14.2	150
Magnesium (Mg)	1.9	80
Manganese (Mn)	<1.0	50
Zinc (Zn)	-	4.0
Other parameters	Result	
рН	6.8	≥ 6,5 и ≤ 9,5

Table 10. Dolnata Chesma Mountain Spring Water, Teteven, Bulgaria

Controlled parameters of ions	Result and measuring unit (mg /dm³)	Maximum limit value (mg /dm ³)
Sodium (Na)	2.5	200
Potassium(K)	1.5	-
Calcium(Ca)	94.4	150
Magnesium (Mg)	12.1	80
Manganese, (Mn) µg/dm ³	1.6	50
Zinc, (Zn)	0.2	4.0
Other parameters	Result	
pH	8.98	≥ 6,5 and ≤ 9,5

Combination of two waters	Calcium (Ca) (mg /dm³)	Sodium (Na) (mg /dm³)	Potassium (K) (mg /dm³)
Velingrad&Bachkovo	74.7	94.2	4.1
Bankia&Bachkovo	87.3	188.4	4.3
Hisar&Bachkovo	76.9	120.6	3.3

Table 11. Mineral contents of combined Velingrad, Bankia, Hisar and Bachkovo water intake

Table 12. Mineral contents of combined *Devin* and *Rilana* water intake

Combination of two waters	Calcium (Ca) (mg /dm³)	Sodium (Na) (mg /dm³)	Potassium (K)
Devin&Rilana	20.3	9.8	-

3.5 Use of Vitamins During Recovery

Bulgaria is rich in herbs. Herbs and plants are usually used for various treatments in sports medicine. Pharmaceutical companies utilize them to prepare liquids from individual herbs, tablets and extracts.

The following herbs and plants are generally used as sources of vitamins in Bulgaria [24-33].

Vitamin E

Oleum Gossypyi Oleum Helianthi Oleum Maydis

Plants synthesize provitamin A.

Urtica dioica L Daucus carota L Spinacia oleracea L

Vitamin C

Rosa canina L Ribes nigrum L Taraxacum officinale

Vitamin B₁

Zea mays L Spinacia oleracea L

Vitamin B₉, folic acid

Urtica dioica L

4. CONCLUSION

Common hydration modalities in sports can be improved by taking into account water composition. Combined intake of different bottled mineral waters was shown to enhance the hydration reaction of Bulgarian athletes during training, competitions and recovery. Our work also represents a pioneering application of electrochemically activated Catholyte water, especially in the recovery stage, resulting in activation of ATP-related biochemical processes and mitochondrial functioning. It should be pointed out that efficient hydration has an extra benefit of reducing travel fatigue in modern sports. In addition, we studied the possibilities of adding natural vitamins to drinking water that are derived from plants and herbs used in Bulgaria. Along these lines, future research will be dedicated to elaboration of hydration modalities of Bulgarian football players based on combined intake of bottled mountain spring and mineral waters together with Catholyte electrochemically activated water.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancementof knowledge. In addition, the research has not been funded by any production company an is a result ofauthors' personal efforts.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Mihail Iliev (Bulgarian Football Union), Valentin

Velikov, Ivaylo Yakimov (PFC Ludogorets), Vihren Bachev, Nezabravka Gencheva, Todor Marinov (National Sports Academy).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Watson PE, Watson ID, Batt RD. Total body water volumes for adult males and females estimated from simple anthropometric measurements'. In: The American Journal of Clinical Nutrition. 1980;33(1):27–39.
- Coyle E. Physiological determinants of endurance exercise performance. In: Journal and Science in Medicine in Sport. 1999;2(3):181-189.
- McCallum Sh. Muscle fatigue, muscle recovery and How this knowledge applies to rock climbers. – In: DJUB. 2017;4.
- Ignatov I, Gluhchev G, Karadzhov S, Ivanov N, Mosin OV. Preparation of electrochemically activated water solutions (catholyte/ anolyte) and studying their physical-chemical properties. In: Journal of Medicine, Physiology and Biophysics. 2015;16:1-14.
- Ignatov I, Karadzhov S, Gluhchev G, Yakimov I. Electromagnetically activated water - properties and effects. In: Bulgarian Journal of Public Health. 2018;10(4):63-69.
- Ignatov I, Gencheva N, Marinov T, Yaneva I, Angelcheva M, Dinkov G, Angushev I. Electrochemically activated water catholyte for the activation of hydrogen ions and atp for sport's shape and recovery. In: Journal of Advances in Medicine and Medical Research. 2020; 32(4):112-119.
- Bachir VM, Pogorelov AG. Universal electrochemical technology for environmental protection. International Journal of Pharmaceutical Research & Allied Sciences. 2018;7(1):41-57.
- Certificate No 2369/1.11.2019, Licensed Eurotest laboratory, Catholyte water, Kangen device, ET Ignat Ignatov, Prof. Ignat Ignatov, Ordinance No 9/2001, Official State Gazette, about the quality of water, intended for drinking purposes, Bulgaria. 2004;30:178.
- 9. Yakimov I. Trends of changes in cardiovascular parameters in football

players in a state of dehydration. Medicine and Sport. 2014;1-2:34-38.

- Yakimov I, Ignatov I. Hydration and properties of water in football for excellent shape, Int. Conference on Medicine and Football, Sofia, Bulgaria, Journal of Medicine and Sport. 2019; 46-51.
- Ignatov I. Electrochemically activated water Catholyte for activation of shape and recovery in the sport. Int. Conference on Medicine and Football, Sofia, Bulgaria. Journal of Medicine and Sport. 2019;3-4:30-34.
- Montain S. Hydration recommendation for sport. Current Sports Medicine. 2008;7(4):187-192
- 13. Duvillard S. Fluids and hydration in prolonged endurance performance. Nutrition. 2004;20(7-8):651-656.
- 14. Paik IY, et al. Fluid replacement following dehydration reduces oxidative stress during recovery'. In: Biochem Biophys Commun. 2009;383(1):103-107.
- 15. Ignatov I, Mosin OV, Naneva K. Water in the human body is information bearer about longevity, Euromedica, Hanover. 2012;110-111.
- Ignatov I, Mosin O V, Bauer E. Effects of Zinc and Manganese in mountain and glacier water for predominant antioxidant effects. Journal of Medicine, Physiology and Biophysics. 2016;27:11-29.
- 17. Ignatov I. Research of the factors of health and longevity of for the population in Bulgaria. Bulgarian Journal of Public Health. 2018;10(3):52-65.
- Ignatov I, Pesheva Y. Studying of the factors of longevity in Smolyan municipality, Rhodope mountains, Bulgaria as area of oxidant/antioxidant balance, Journal of Natural Sciences Research. 2018;8(6):29-42.
- 19. Urticae herba Urtica dioica. European Medicines Agency Evaluation of Medicines for Human Use. Doc. Ref. EMEA/HMPC/366106/2007.
- 20. Zahariev P, Boycheva P, Kosev K. Review on the medicinal plants of the north black sea coast, Annual of Sofia University St. Kliment Ohridski; 2016.
- Ignatov I. (2020) Physicochemical Research of Mineral and Mountain Spring Waters in Bulgaria, Asian Journal of Applied Chemistry Research. 2020; 7(2): 40-46.
- 22. Ignatov I. Standards for the Research and Registration of Drinking Mineral and

Mountain Spring Waters in Bulgaria. Asian Journal of Chemical Sciences. 2021; 9 (1): 12-18.

- Ignatov I. Microbiological Research of Mineral and Mountain Spring Waters in Bulgaria, Microbiology Research Journal International. 2020; 30 (10): 79-84.
- Yaneva I, Balabanski V. Traditional herbal therapy for some cardiovascular diseases in Bulgaria. European Journal of Medicine. 2019;7(1):45-57.
- Dar S. Bioassay guided isolation and identification of anti-inflammatory and antimicrobial compounds from *Urtica dioica* L. (Urticaceae) leaves. African Journal of Biotechnology. 11 (65).
- Ghaima K, Hashim N, Safaa A. Antibacterial and antioxidant activities of ethyl acetate extract of nettle (Urtica dioica) and dandelion (Taraxacum officinale). Journal of Applied Pharmaceutical Science. 2013;3(5):96-99.
- Manganelli R, Zaccaro L, Tomei P. Antiviral activity in vitro of Urtica dioica L., Parietaria diffusa M. et K. and Sambucus nigra L. J Ethnopharmacol. 2005;98(3):323-7
- Bachev V, Yaneva-Balabanska I, Plantbased food supplements: Bulgarian and European legalization. Bulgarian Journal of Public Health. 2018;10(1):53-66.

- Yaneva I, Balabanski V, Karanesheva T, Ignatov I. Some endangered healings plants in Bulgaria – Legislative regulation, Protection, Characteristic description, Application, Agricultural cultivation. Bulgarian Journal of Agricultural Science. 2020;26(4):847-852.
- Krastev D, Ignatov I, Mosin OV, Penkov P. Research on the effects of the 'Dance of the Spiral' Methodology, with spectral analysis of water extracts, upon the physiological parameters of plants and the essential oil content, Advances in Physics Theories and Applications. 2016;52: 47-55.
- Yaneva I, Balabanski V, Plant used in dyslipidemias in conventional and unconventional medicine. Bulgarian Journal of Public Health, 2015;7(3):13-34.
- 32. Balabanski V, Yaneva I. Application of medicinal products influencing on renninangiotensin-aldosteron-system at intermediary health risk factor of high blood pressure. Bulgarian Journal of Public Health. 2015;7(4):14-33.
- Ignatov I, Popova T. Applications of Moringa oleifera, Urtica dioica, Malva sylvestris and Plantago major Containing Potassium for Recovery. Plant Cell Biotechnology and Molecular Biology Journal. 2021;22(7-8):93-103.c

© 2021 Ignatov et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/66181