

ARRHENIUS KINETICS IN THE EVALUATION OF THE BIOLOGICAL ACTIVITY OF PHARMACEUTICAL TINCTURES

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ABSTRACT

Objective: To develop a method for identifying tinctures by assessing their biological activity at different temperatures based on Arrhenius kinetics.

Methods: The tinctures of anti-inflammatory and cardiotoxic actions were chosen for the Spirotox-test. Chromatographic ethanol (HPLC grade, 99.8%, Fisher Scientific, UK) and deionized high-resistance water (18.2 MΩ×cm, Milli-Q, Millipore) were used to prepare 70% water-alcohol extractant. The dispersity of the infusions and solvents was evaluated by LALLS (Malvern, UK) (micrometer range) and DLS (nanometer range) (Zetasizer Nano ZS, Malvern, UK) methods.

Results: The observed (obs) values of activation energy (${}^{obs}E_a$) of ligand-receptor interactions of infusoria *Spirostomum ambiguum* and components of tinctures with regard to the extractant: water volume ratio was ranked in descending order of toxicity: motherwort (1:10; 87±13 kJ/mol)>calendula (1:7; 103±18 kJ/mol)>eucalyptus (1:7; 159±5 kJ/mol)>valerian (1:5; 135±6 kJ/mol)>hawthorn (1:4; 113±20 kJ/mol). The found values of activation energy were included in the previously created library for the construction of the correlation diagram « ${}^{obs}E_a$ -LD₅₀», which allowed to assess the toxicity of tinctures in comparison with other pharmaceutical substances.

Conclusion: The method for assessing the biological activity of tinctures was developed by Arrhenius kinetics. The values of activation energy ${}^{obs}E_a$ of ligand-receptor interactions can be used for the identification of tinctures.

Keywords: Tinctures, Spirotox-test, Arrhenius kinetics, Activation energy, LALLS, DLS, Electrokinetic potential

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INTRODUCTION

According to the World Health Organization, about 80% of the world's population uses traditional medicines, including plant drugs, before giving preference to synthetic medicines [1]. Medicinal plants are a rich source of biologically active compounds and are used to prepare various dosage forms, including tinctures [2-4]. According to the pharmacopeias of different countries, to control the quality of tinctures, the color, density, and mass of the dry residue after removal of the extractant are determined [5, 6]. Ideally, quality control of tinctures can be carried out by chromatographic method for a specific component, which, for example, for valerian tincture are valerianic and acetoxyvalerianic acids [7, 8]. But for most tinctures, individual components are only partially identified, and standard samples corresponding to them have not been developed. It is precisely because of the lack of Chemical Reference Substances (CRS) that there are practically no private articles on individual tinctures. The variety of tinctures on the pharmaceutical market requires the improvement of methods for controlling their quality, including the determination of authenticity based on physical and chemical methods [9-12].

In this article, for the first time, a biological testing method for the identification of tinctures is proposed. For bio testing, the infusoria *Spirostomum ambiguum* (*Sp. ambiguum*), is a reliable tool for determining the biological activity of pharmaceutical substances based on Arrhenius kinetics [13].

The purpose of the study is to develop a method for identifying tinctures by assessing their biological activity at different temperatures based on Arrhenius kinetics.

MATERIALS AND METHODS

Tinctures from different manufacturers of two pharmacological groups were studied. The first of them is represented by tinctures of antiseptic and anti-inflammatory action-calendula (*Calendula officinalis, flores*) and eucalyptus (*Eucalyptus globulus, folium*), the

second-tinctures of hypotensive and cardiotoxic action-valerian (*Valeriana officinalis, rhizomata cum radicibus*), motherwort (*Leonurus spp., herba*), and hawthorn fruit (*Crataegus spp., fructus*).

The Spirotox-test was carried out on an equipment including a five-well glass plate with a thermostatically controlled water jacket (Lauda-Königshofen, Germany). The biosensor *Sp. ambiguum* observation was carried out using a binocular microscope MBR-10 (Altami, Saint Petersburg, Russia). The rate constant of the ciliates death $k=1/t_{life}$ was determined at 5 temperatures in the range from 22 °C to 30 °C. Lifespan t_{life} was recorded from the moment of incubation to death (complete immobilization or rupture of the cell membrane). Linearization in semi-logarithmic coordinates "lnk-1/T" allowed to calculate the angle of inclination (α) to the abscissa axis. According to the Arrhenius equation, the value of the observed activation energy of the ciliates death process was calculated: $E_a=R \times t_{g\alpha}$.

The biological activity of the tinctures was compared with 70% ethanol prepared by dilution of absolute chromatographic ethanol (99.8%, Fisher Scientific, UK) with deionized high-resistance water (18.2 MΩ×cm at 25 °C; Milli-Q system-Millipore, Great Britain). The concentration of the water-ethanol extractant was controlled with an alcohol hydrometer.

For the study of *Sp. ambiguum* death rate, the initial tinctures and prepared 70% ethanol were diluted with high-resistance water in different ratios of 1:3 to 1:10 by volume and mixed with light shaking. The presence of the dispersed phase particles in the obtained mixtures was controlled by low-angle laser light scattering (LALLS, Malvern, UK) in the micrometer range and by dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern, UK) in the nanometer range.

PASS Online program was used to demonstrate the biological properties of the tinctures components [14-16]. To assess the relationship between the structure of the compound and pharmacological activity, the values of the probability fraction P_a in the range from 0.800 to 1.000 were used.

The results were statistically processed and plotted using the OriginPro 2021 program (OriginLab, USA).

RESULTS AND DISCUSSION

Components of medicinal plants and their specific biological activity

Studies of the biological activity of tinctures were preceded by an analysis of information on the components of medicinal plants.

The raw material from which the studied tinctures were prepared contains compounds of different chemical classes that determine therapeutic effects (table 1). For example, aqueous ethanol extracts of eucalyptus leaves (*Eucalyptus globulus, folium*) may contain terpenes (cineol, eucalyptol, terpineol, eudesmol), flavonoids (eucalyptin, quercetin, kaempferol), coumarols (coumarin, herniarine, scopoletin, daphnoretin) [17]. *Calendula officinalis, flores* contains carotenoids, such as lutein, as well as a wide range of flavonoids, terpenes, water-soluble glycosides, and volatile esters [18].

Table 1: Some examples of chemical components of tinctures and their pharmacological properties according to PASS online [14-16]

S. No.	Tinctures of	Chemical classification of components	Pharmacological properties
1	<i>Calendula officinalis, flores</i> <i>Eucalyptus globulus, folium</i>	<p>Coumarins</p> <ul style="list-style-type: none"> Scopoletin <p>7-hydroxy-6-methoxychromen-2-one</p> <p>Carotenoids</p> <p>Lutein</p> <p>(1R)-4-[(1E,3E,5E,7E,9E,11E,13E, 15E,17E)-18-[(1R,4R)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethylcyclohex-3-en-1-ol</p> <p>Terpenes and Terpenoids</p> <p>Arnidiol (Arnidenediol)</p> <p>(3S,4aR,6aR,6aR,6bR,8S,8aS,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,12,14b-heptamethyl-11-methylidene-1,2,3,4a,5,6,6a,7,8,9,10,12,12a,13,14,14a-hexadecahydricene-3,8-diol</p> <p>α-Terpineol</p> <p>2-(4-methylcyclohex-3-en-1-yl)propan-2-ol-menth-1-en-8-ol</p> <p>Flavonoids and glycosides</p> <p>Kaempferol</p> <p>3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one</p> <p>Rutin</p> <p>2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[[2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy]chromen-4-one</p>	<p>0.900 Membrane integrity agonist</p> <p>0.898 Antimutagenic</p> <p>0.890 Cardiovascular analeptics</p> <p>0.824 Urinary spasmolytic</p> <p>0.913 Anticarcinogenic</p> <p>0.895 Radioprotector</p> <p>0.892 Dermatological</p> <p>0.877 Keratolytic</p> <p>0.864 Antipsoriatic</p> <p>0.960 Anticarcinogenic</p> <p>0.898 Hepatoprotector</p> <p>0.837 Anti-eczema</p> <p>0.862 Respiratory analeptic</p> <p>0.837 Carminative</p> <p>0.825 Anti-eczema</p> <p>0.804 Analeptic</p> <p>0.974 Membrane integrity agonist</p> <p>0.948 Antimutagenic</p> <p>0.894 Antihemorrhagic</p> <p>0.852 Antiseborrheic</p> <p>0.814 Cardioprotector</p> <p>0.807 Vasoprotector</p> <p>0.993 Hemostatic</p> <p>0.984 Membrane integrity agonist</p> <p>0.983 Anticarcinogenic</p> <p>0.980 Vasoprotector</p> <p>0.968 Hepatoprotective agent</p> <p>0.923 Antioxidant</p> <p>0.907 Antiprotozoal (leishmania)</p> <p>0.939 Phobic disorders treatment</p> <p>0.857 Membrane integrity agonist</p> <p>0.834 Vasoprotector</p> <p>0.997 Antiemetic</p> <p>0.995 Antidepressant</p> <p>0.994 Mood disorders treatment</p> <p>0.993 Antipsychotic</p> <p>0.989 Membrane integrity agonist</p> <p>0.987 Hemostatic</p> <p>0.984 Cardioprotectant</p> <p>0.947 Vasoprotector</p>
2	<i>Valeriana officinalis, rhizomata cum radicibus</i> <i>Leonurus spp., herba</i> <i>Crataegus spp., fructus</i>	<p>Carboxylic acids</p> <p>Valeric acid</p> <p>pentanoic acid</p> <p>Alkaloids</p> <p>Stachydrine</p> <p>(S)-2-carboxylato-1,1-dimethylpyrrolidinium</p> <p>Glycosides</p> <p>Hyperoside</p> <p>3-(β-D-Galactopyranosyloxy)-3',4',5,7-tetrahydroxyflavone</p>	<p>0.939 Phobic disorders treatment</p> <p>0.857 Membrane integrity agonist</p> <p>0.834 Vasoprotector</p> <p>0.997 Antiemetic</p> <p>0.995 Antidepressant</p> <p>0.994 Mood disorders treatment</p> <p>0.993 Antipsychotic</p> <p>0.989 Membrane integrity agonist</p> <p>0.987 Hemostatic</p> <p>0.984 Cardioprotectant</p> <p>0.947 Vasoprotector</p>

Extracts of rhizomes and roots of valerian (*Valeriana officinalis, rhizomata cum radicibus*) are among the most popular herbal preparations and also contain compounds of the same classes [19]. A detailed study of the mechanisms of valerian tincture action showed that its sedative effect is due to the presence of valerenic acid and valerenol, which selectively interact with glutamate receptors in the body [20]. The cardioprotective potential of ursolic acid and the alkaloid stahydrin of motherwort grass (*Leonurus spp., herba*) is well known, but this plant also contains numerous polyphenolic compounds (quercetin, hyperoside, rutin), triterpene acids, amines, and carboxylic acids [21-23]. The fruits of hawthorn (*Crataegus spp., fructus*) are no exception, in which polyphenols have been identified [24, 25].

Despite the uniformity of the composition of medicinal plants, each type of plant may contain the same compounds but in different quantities. That is why it is possible to predict the different biological activity of tinctures, despite the identity of the method of their manufacture.

Bioactive compounds present in tinctures are involved in ligand-receptor interactions at the cellular level of the body. Using infuzoria *Sp. ambiguum* makes it possible to generalize the mechanisms of

such interactions, presenting them in the form of a well-known scheme [13]:



This approach allows us to evaluate the total biological activity of the components of each tincture in Arrhenius coordinates through ${}^{\circ}\text{bsE}_a$ -the activation energy of cell death.

Tinctures as dispersed systems

To assess the possibility of influencing the ligand-receptor interactions of particles of the dispersed phase, we studied the dispersion of tinctures. It is known that micron-sized clusters of deuterium-stabilized water are formed in aqueous solutions [23]. The size spectra of water-ethanol clusters in the micrometer range also demonstrate polymodality with three size groups: 9–12 μm , 20–25 μm , and 40–50 μm . The polydispersity of alcohol dilutions is also observed in the nanorange (fig. 1). At the same time; water dilutions are characterized by the presence of a stable band with a maximum of about 100 nm on the size spectrum, regardless of the species differences of the tincture. Similar results were obtained earlier for aqueous solutions of motherwort tinctures [24].

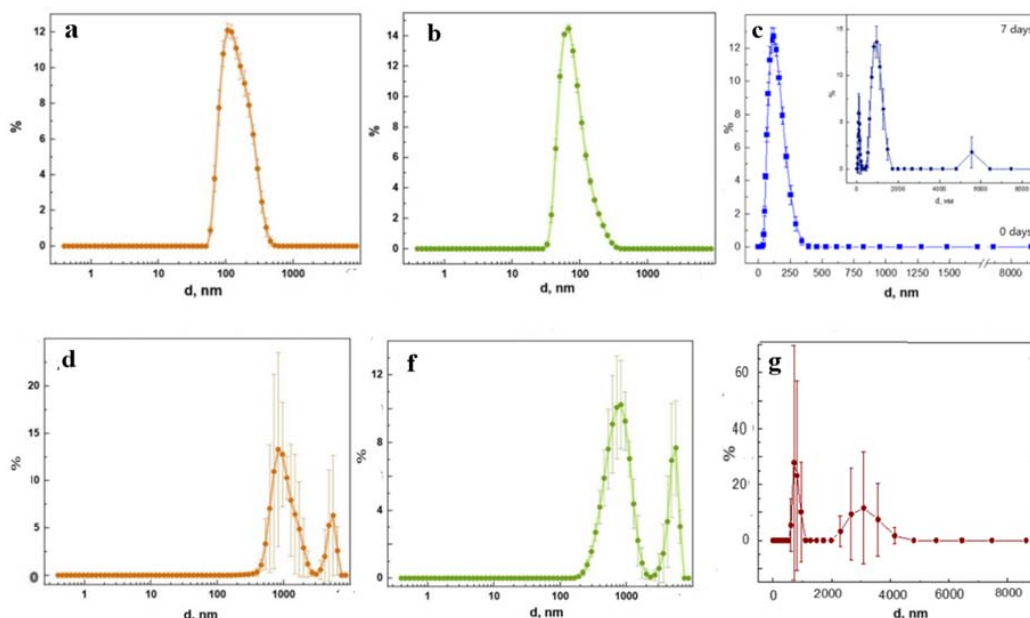


Fig. 1: Size spectra of dynamic light scattering (DLS) of freshly prepared water (a, b, c) and alcohol (d, f, g) dilutions (1:40) of calendula (a, d), eucalyptus (b, f), valerian (c, g) tinctures. Insert in fig. 1c-size spectrum in 7 d. The results are presented as mean \pm SD, n=5

The electrokinetic (ζ) potential measurement also indicates an increase in stability when the proportion of ethanol in the disperse system decreases. For example, the average values of the zeta potential for aqueous dilution of calendula tincture were (-33.7 ± 2.2) mV, which exceeded the boundary value of the stability of colloidal systems [12, 28, 29]. On average, for all tinctures, the zeta potential of the particles of the dispersed phase in aqueous dilutions was 1.5 times higher than alcohol dilutions. The stable nanoparticles found in aqueous dilutions can play the role of carriers of biologically active components into the cell, for example, by pinocytosis. Thus, aqueous dilutions of tinctures are

characterized by low dispersion and high stability, which made it possible to study their biological activity using the Spirotox method.

Assessment of the biological activity of tinctures

The use of the Arrhenius coordinates " $\ln k - 1/T$ " made it possible to obtain the values of activation energies for the death of *Sp. ambiguum* for tinctures of different pharmacological classes. As it turned out, higher values of ${}^{\circ}\text{bsE}_a$ (lower toxicity) are characteristic of the extractant (70% ethanol) in the same volumetric dilutions that were chosen for tinctures (table 2).

Table 2: Activation energies of *Sp. ambiguum* death in tinctures and water-ethanol mixtures, (mean \pm SD; n = 3, P = 0.95)

Tincture	Tincture dilution ratio	Activation energy of <i>Sp. ambiguum</i> death, ${}^{\circ}\text{bsE}_a \pm \text{SD}$, kJ/mol	
		tincture	diluted ethanol
Hawthorn	1:4	113 \pm 20	125 \pm 17
Valerian	1:5	135 \pm 6	143 \pm 10
Eucalyptus	1:7	159 \pm 5	258 \pm 8
Calendula	1:7	103 \pm 18	258 \pm 8
Motherwort	1:10	87 \pm 13	-($t_{\text{life}} > 60 \text{ min}$)

The presence of various organic and inorganic components in tinctures enhances their toxic effect. This is manifested in the lifetime (t_{life}) decreasing, in the rate constant ($k=1/t_{life}$) increasing, and in lower values of the activation energy. For example, the lowest activation energy was obtained for motherwort tincture ($^{obs}E_a=87\pm 13$ kJ/mol) at the maximum dilution with water (1:10). Motherwort tincture is the most toxic in the study group of tinctures. At the same time, for tinctures of calendula and eucalyptus, the same dilution ratio (1:7) was required, but the average value of activation energy differs statistically significantly for eucalyptus (159 kJ/mol) and calendula (103 kJ/mol). That is why calendula tincture is more toxic than the tincture of eucalyptus. Despite the absence of statistically significant differences in the activation energies of valerian and hawthorn tinctures, the latter is considered less toxic, since it has a lower dilution with water (1:4) in the experiment.

The observed values of the activation energy of ligand-receptor interactions and the dilution factor made it possible to rank the tinctures in order of decreasing toxicity: motherwort (1:10; 87 ± 13 kJ/mol) > calendula (1:7; 103 ± 18 kJ/mol) > eucalyptus (1:7; 159 ± 5 kJ/mol) > valerian (1:5; 135 ± 6 kJ/mol) > hawthorn (1:4; 113 ± 20 kJ/mol).

The multiplicity of dilution and the value of $^{obs}E_a$ allow us to recommend a method for determining the authenticity of tinctures.

The position of tinctures in diagram $^{obs}E_a$ -LD₅₀

The found activation energy values were included in the previously created library for constructing the $^{obs}E_a$ -LD₅₀ correlation diagram, which made it possible to evaluate the toxicity of tinctures in comparison with other pharmaceutical substances. Data on semi-lethal doses of tinctures, when administered to animals, are limited. It is known that LD₅₀ after a single intraperitoneal injection of an alcohol extract of valerian roots to mice is 3300 mg/kg [30]. A study of the hawthorn preparation (an extract from hawthorn leaves and flowers in 45% ethanol in a mass ratio of 6:1) showed that its single administration at a dose of 3 g/kg orally and intraperitoneally to rats and mice did not cause the death of animals [25]. There are no data on the assessment of the toxicity of tinctures of eucalyptus and motherwort on laboratory animals [15, 22]. The « $^{obs}E_a$ -LD₅₀» diagram, developed earlier [13] and supplemented by the results of the determination, makes it possible to predict the toxicity of tinctures based on the $^{obs}E_a$ values found (fig. 2).

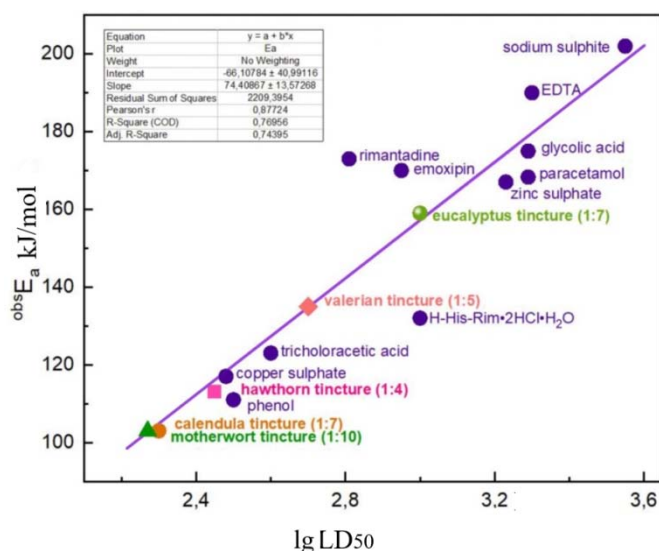


Fig. 2: Diagram of mutual correspondence between the activation energy of the biosensor cell death $^{obs}E_a$ and LD₅₀ of laboratory animals (rats, per os). LD₅₀ in mg/kg

The inclusion of the found values of activation energy in the previously created library for constructing the correlation diagram « $^{obs}E_a$ -LD₅₀» makes it possible to evaluate the toxicity of any tincture in comparison with pharmaceutical substances without conducting an economically costly and ethically limited experiment with mammalian animals.

CONCLUSION

For the first time, the Spirotox-test method was applied to develop a methodology for assessing the biological activity (toxicity) of tinctures using Arrhenius kinetics. The values found for the activation energy of *Sp. ambiguus* death in aqueous dilutions of tinctures of different multiplicity allow to determine the authenticity of tinctures.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

- World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023. Geneva: World Health Organization. 2013. p. 1-76. Available from: <https://apps.who.int/iris/handle/10665/92455>. [Last accessed on 06 May 2023]
- Nath R, Kityania S, Nath D, Talkudar AD, Sarma G. An extensive review on medicinal plants in the special context of economic importance. Asian J Pharm Clin Res. 2023;16(2):6-11. doi: 10.22159/ajpcr.2023.v16i2.46073.
- Rahul G, Nandhakumar E. A systematic review of five herbal ingredients for the management of diabetes mellitus. Asian J Pharm Clin Res. 2022;15(12):18-23. doi: 10.22159/ajpcr.2022.v15i12.45427.
- Ahmad F, Kamaraj B, Mohamed JMM, Ebrahim D, Al Humoud SY, El-sagheer AM. Ethnopharmacological and phytochemical study of major species from labiatae family in Saudi Arabia: a systematic review. Int J App Pharm 2022;1:44-57. doi: 10.22159/ijap.2022.v14ti.40.
- European Pharmacopoeia 11.2. Strasbourg: EDQM Council of Europe; 2023. Available from: <https://online.uspnf.com/uspnf>. [Last accessed on 06 May 2023]
- United States Pharmacopoeia. Rockville (MD): United States Pharmacopoeial Convention, USA. Vol. 2022;2(3). Available from: <https://pheur.edqm.eu/subhome/11> [cited May 6 2023].

7. Shinjyo N, Waddell G, Green J. Valerian root in treating sleep problems and associated disorders-a systematic review and meta-analysis. *J Evid Based Integr Med.* 2020;25:2515690X20967323. doi: 10.1177/2515690X20967323, PMID 33086877, PMCID 7585905.
8. European Pharmacopoeia 11.2. Strasburg: EDQM Council of Europe. Valerian Tincture. 2023. p. 1771-2. Available from: <https://online.uspnf.com/uspnf>. [Last accessed on 06 May 2023]
9. Lunz K, Stappen I. Back to the roots-an overview of the chemical composition and bioactivity of selected root-essential oils. *Molecules.* 2021;26(11). doi: 10.3390/molecules26113155. PMID 34070487.
10. Zhang RH, Liu ZK, Yang DS, Zhang XJ, Sun HD, Xiao WL. Phytochemistry and pharmacology of the genus *Leonurus*: the herb to benefit the mothers and more. *Phytochemistry.* 2018;147:167-83. doi: 10.1016/j.phytochem.2017.12.016. PMID 29335190.
11. Wardana MS, Jufri M, Munim A. Physicochemical properties and nutrition of moringa oleifera lam. leaf extract: a preliminary study on preparation phytosomes as herbal supplement for children. *Int J App Pharm.* 2022;14(1):281-7. doi: 10.22159/ijap.2022v14i1.43477.
12. Maurya H, Kumar T. Formulation, standardization, and evaluation of polyherbal dispersible tablet. *Int J App Pharm.* 2019;11(1):158-67. doi: 10.22159/ijap.2019v11i1.30113.
13. Goncharuk VV, Syroeshkin AV, Zlatskiy IA, Uspenskaya EV, Orekhova AV, Levitskaya OV. Quasichemical description of the cell death kinetics of cellular biosensor *Spirostomum ambigua* for testing the biological activity of aqueous solutions. *J Water Chem Technol.* 2017;39(2):97-102. doi: 10.3103/S1063455X17020072.
14. Way2Drug. Available from: <http://www.way2drug.com/passonline/>. Last 06 May 2023]
15. Duc Nguyen HD. Neurotherapeutic effects of quercetin and its metabolite compounds on cognitive impairment and Parkinson's disease: an *in silico* study. *Eur J Drug Metab Pharmacokinet.* 2023;48(2):151-69. doi: 10.1007/s13318-023-00816-w, PMID 36848007.
16. Merlani M, Nadaraia N, Amiranashvili L, Petrou A, Geronikaki A, Ciric A. Antimicrobial activity of some steroidal hydrazones. *Molecules.* 2023;28(3):36770834. doi: 10.3390/molecules28031167, PMID 36770834, PMCID PMC9920613.
17. European Medicines Agency. Committee on Herbal Medicinal Products, European Union. Addendum to Assessment report on *Eucalyptus globulus Labill. folium*; 2022. Available from: <https://www.ema.europa.eu/en/medicines/herbal/eucalypti-folium>. [Last accessed on 06 May 2023]
18. European Medicines Agency. Committee on Herbal Medicinal Products, European Union. Assessment report on *Calendula officinalis L., flos*; 2018. Available from: <https://www.ema.europa.eu/en/medicines/herbal/calendulea-e-flos>. [Last accessed on 06 May 2023]
19. Patan A. *Valeriana jatamansi*: an ethnobotanical review. *Asian J Pharm Clin Res.* 2018;11(16):38-40. doi: 10.22159/ajpcr.2018.v11s4.31706.
20. Das G, Shin HS, Tundis R, Gonçalves S, Tantengco OAG, Campos MG. Plant species of sub-family valerianaceae-a review on its effect on the central nervous system. *Plants (Basel).* 2021;10(5):846. doi: 10.3390/plants10050846, PMID 33922184.
21. European Medicines Agency. Committee on Herbal Medicinal Products, European Union. Assessment report on *Leonurus cardiaca L., herba*; 2010. Available from: <https://www.ema.europa.eu/en/medicines/herbal/leonuricardiaca-herba>. [Last accessed on 06 May 2023]
22. Angeloni S, Spinozzi E, Maggi F, Sagratini G, Caprioli G, Borsetta G. Phytochemical profile and biological activities of crude and purified *Leonurus cardiaca* extracts. *Plants (Basel).* 2021;10(2):195. doi: 10.3390/plants10020195, PMID 33494336.
23. European Medicines Agency. Committee on Herbal Medicinal Products, European Union. Addendum to Assessment report on *Leonurus cardiaca L. herba*; 2019. Available from: <https://www.ema.europa.eu/en/medicines/herbal/leonuricardiaca-herba> [Last accessed on 06 May 2023]
24. Nazhand A, Lucarini M, Durazzo A, Zaccardelli M, Cristarella S, Souto SB. Hawthorn (*Crataegus spp.*): an updated overview on its beneficial properties. *Forests.* 2020;11(5):564. doi: 10.3390/f11050564.
25. European Medicines Agency. Committee on Herbal Medicinal Products, European Union. Assessment report on *Crataegus spp.*; 2016. Available from: <https://www.ema.europa.eu/en/medicines/herbal/crataegifolium-cum-flore>. [Last accessed on 06 May 6]
26. Syroeshkin AV, Pleteneva TV, Uspenskaya EV, Zlatskiy IA, Antipova NA, Grebennikova TV. D/H control of chemical kinetics in water solutions under low deuterium concentrations. *Chem Eng J.* 2019;377:119827. doi: 10.1016/j.cej.2018.08.213.
27. Syroeshkin AV, Ogotoeva DD, Galkina DA, Uspenskaya EV, Pleteneva TV. Polarimetry and dynamic light scattering in quality control of cardiotoxic and hypotensive tinctures. *Int J App Pharm.* 2022;14(6):114-9. doi: 10.22159/ijap.2022v14i6.45907.
28. Clogston JD, Patri AK. Zeta potential measurement. *Methods Mol Biol.* 2011;697:63-70. doi: 10.1007/978-1-60327-198-1_6, PMID 21116954.
29. Ferreyra Maillard APV, Espeche JC, Maturana P, Cutro AC, Hollmann A. Zeta potential beyond materials science: applications to bacterial systems and to the development of novel antimicrobials. *Biochim Biophys Acta Biomembr.* 2021;1863(6):183597. doi: 10.1016/j.bbmem.2021.183597. PMID 33652005.
30. European Medicines Agency. Committee on Herbal Medicinal Products, European Union. Assessment report on *Valeriana officinalis L., radix* and *Valeriana officinalis L.*; 2016. Available from: <https://www.ema.europa.eu/en/medicines/herbal/Valeriana-radix>. [Last accessed on 06 May 2023]