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 valerenic and acetoxyvalerenic acids [7, 8]. But for most tinctures, individual components are only partially identified, and 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 infusion 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, rhizoma cum radicibus), motherwort (Leonurus spp., herba), and hawthorn fruit (Crataegus spp., fructus).

The Spirostom-test was carried out on an equipment including a five-channel photomultiplier Sp. 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±60 kJ/mol). The found values of activation energy were included in the previously created library for the construction of the correlation diagram "obsEa-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 obsEa of ligand-receptor interactions can be used for the identification of tinctures.

Keywords: Tinctures, Spirostox-test, Arrhenius kinetics, Activation energy, LALLS, DLS, Electrokïnetic potential

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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 cardiotonic actions were chosen for the Spirostox-test. Chromatographic ethanol (HPLC grade, 99.8%, Fisher Scientific, UK) and deionized high-resistance water (18.2 MΩcm, Milli-Q, Millipore) were used to prepare 70% water-alcohol extractant. The dispersity of the infusion 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 (obsEa) of ligand-receptor interactions of infusion 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±60 kJ/mol). The found values of activation energy were included in the previously created library for the construction of the correlation diagram "obsEa-LD50", which allowed to assess the toxicity of tinctures in comparison with other pharmaceutical substances.

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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, terpinel, 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]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Tinctures of medicinal plants</th>
<th>Chemical classification of components</th>
<th>Pharmacological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calendula officinalis, flores Eucalyptus globulus, folium</td>
<td>Coumarins</td>
<td>0.900 Membrane integrity agonist 0.898 Antimutagenic 0.890 Cardiovascular analeptics 0.824 Urinary spasmolytic</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Scopoletin 7-hydroxy-6-methoxychromen-2-one</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Lutênio 1R-4-[[E,3,E,5,E,7,E,9,E,11,E,13,E,15,E,17]-18-[(1R,4R)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-yl]-3,7,12,16-tetramethylcyclohexa-1,3,5,7,9,11,13,15,17-no naenyl]-3,5,5-trimethylcyclohex-3-en-1-ol</td>
<td>Terpenes and Terpenoids 0.913 Anticarcinogenic 0.895 Radioprotector 0.892 Dermatological 0.877 Keratolytic 0.864 Anti-eczema</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Arnidiol (Arnidenedioi) (35,4aR,6aR,6bR,8S,8aS,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,12,14-biotheptamethyl-1,R-methylidene-2,2,3,4a,5,6,6a,7,8,9,10,12a,13,14,14a,hexadecadecahydropicene-3,8-diol</td>
<td>Carboxylic acids 0.862 Respiratory analeptic 0.837 Carminative 0.825 Anti-eczema 0.804 Analgetic</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>(\alpha)-Terpinêol 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol-menth-1-en-8-ol</td>
<td>Flavonoids and glycosides 0.974 Membrane integrity agonist 0.946 Antimutagenic 0.984 Antihemorrhagic 0.852 Antiserbohemic 0.814 Cardioprotector 0.807 Vasoprotector</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Kaempferol 3,5,7-trihydroxy-2-{4-hydroxyphenyl}chromen-4-one</td>
<td>0.993 Hemostatic 0.984 Membrane integrity agonist 0.983 Anticarcinogenic 0.980 Vasoprotector 0.968 Hepatoprotective agent 0.923 Antioxidant 0.907 Antiprotozoal (leishmania)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Rutin 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-{25,3R,4S,5S,6R}-3,4,5-trihydroxy-6-{2R,3R,4R,5R,6S}-3,4,5-trihydroxy-6-methoxyl-2-yl</td>
<td>Carboxylic acids 0.939 Phobic disorders treatment 0.857 Membrane integrity agonist 0.834 Vasoprotector</td>
</tr>
<tr>
<td>2</td>
<td>Valeriana officinalis, rhizomata cum radixibus Leonurus spp., herba Crataegus spp., fructus</td>
<td>Carboxylic acids 0.997 Antiemetic 0.995 Antidepressant 0.994 Mood disorders treatment 0.993 Antipsychotic</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Valeric acid pentanoic acid Alkaloids 0.999 Membrane integrity agonist 0.987 Hemostatic 0.984 Cardioprotectant 0.947 Vasoprotector</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Stachydrine (S)-2-carboxylo-1,1-dimethylpyrrolidinium Glycosides 0.989 Membrane integrity agonist 0.987 Hemostatic 0.984 Cardioprotectant 0.947 Vasoprotector</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Hyperoside 3-((\beta)-D-Galactopyranosyloxy)-3',4',5,7-tetrahydroxyflavone</td>
<td></td>
</tr>
</tbody>
</table>
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]:

\[
C \text{(cell)} + nL \xrightarrow{\text{fast}} C \cdot L_n \xrightarrow{\text{slowly}} DC \text{(death)}
\]

This approach allows us to evaluate the total biological activity of the components of each tincture in Arrhenius coordinates through \(\theta_{oc}E_a\), 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].

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(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 \(\theta_{oc}E_a\) (lower toxicity) are characteristic of the extractant (70% ethanol) in the same volumetric dilutions that were chosen for tinctures (table 2).

![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±SD, n=5](image-url)

<table>
<thead>
<tr>
<th>Tincture</th>
<th>Tincture dilution ratio</th>
<th>Activation energy of Sp. ambiguum death, (\theta_{oc}E_a),±SD, kJ/mol</th>
<th>tincture diluted ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawthorn</td>
<td>1:4</td>
<td>113±20</td>
<td>125±17</td>
</tr>
<tr>
<td>Valerian</td>
<td>1:5</td>
<td>135±6</td>
<td>143±10</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>1:7</td>
<td>159±5</td>
<td>258±8</td>
</tr>
<tr>
<td>Calendula</td>
<td>1:7</td>
<td>103±18</td>
<td>258±8</td>
</tr>
<tr>
<td>Motherwort</td>
<td>1:10</td>
<td>87±13</td>
<td>&lt;&lt;(t&lt;60 min)</td>
</tr>
</tbody>
</table>
The presence of various organic and inorganic components in tinctures enhances their toxic effect. This is manifested in the lifetime (t_{\text{life}}) decreasing, in the rate constant (k=1/t_{\text{life}}) increasing, and in lower values of the activation energy. For example, the lowest activation energy was obtained for motherwort tincture (\(159\, \text{kJ/mol}\)) and calendula (103\, \text{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\, \text{kJ/mol}) and calendula (103\, \text{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\, \text{kJ/mol})>calendula (1:7; 103±18\, \text{kJ/mol})>eucalyptus (1:7; 159±5\, \text{kJ/mol})>valerian (1:5; 135±6\, \text{kJ/mol})>hawthorn (1:4; 113±20\, \text{kJ/mol}).

The multiplicity of dilution and the value of \(\text{obs}E_a\) allow us to recommend a method for determining the authenticity of tinctures.

The position of tinctures in diagram \(\text{obs}E_a-LD_{50}\)

The found activation energy values were included in the previously created library for constructing the \(\text{obs}E_a-LD_{50}\) 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 LD_{50} 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 \(\text{obs}E_a-LD_{50}\) diagram, developed earlier [13] and supplemented by the results of the determination, makes it possible to predict the toxicity of tinctures based on the \(\text{obs}E_a\) values found (fig. 2).

**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. ambiguum death in aqueous dilutions of tinctures of different multiplicity allow to determine the authenticity of tinctures.

**FUNDING**

This paper has been supported by the RUDN University Strategic Academic Leadership Program.

**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICT OF INTERESTS**

Declared none

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**REFERENCES**


