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# Antihypoxic Action of *Panax Japonicus*, *Tribulus Terrestris* and *Dioscorea Deltoidea* Cell Cultures: In Silico and Animal Studies

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**Abstract:** Chemical diversity of secondary metabolites provides a considerable variety of pharmacological actions with a significant extension due to their combinations in plant extracts. Production of plant-derived medicinal products in cell cultures has advantages because of the efficient use of different biotic and abiotic elicitors and better control of the developmental processes. Using PASS software, we predicted biological activity spectra for phytoconstituents identified in cell cultures of *Panax japonicus* (12 molecules), *Tribulus terrestris* (4 molecules), and *Dioscorea deltoidea* (3 molecules). Mechanisms of action associated with the antihypoxic effect were predicted for the majority of molecules. PharmaExpert software allowed analyzing possible synergistic or additive effects of the combinations of phytoconstituents associated with the antihypoxic action. Experimental studies of the antihypoxic effect of the plants' extracts in water and ethanol have been performed in 3 animal models: Acute asphyctic hypoxia (AAH), Acute haemic hypoxia (AHeH), and Acute histotoxic hypoxia (AHtH). Effects of *Panax japonicus* and *Tribulus terrestris* preparations exceeded the activity of the reference drug Mexidol in the AHtH model. In the AHeH model, all preparations demonstrated moderate activity; the most potent has been observed for *Dioscorea deltoidea.* Thus, we found that experimental studies in animal models have confirmed the *in silico* prediction.

**Keywords:** natural products · medicinal plant · phytocomponents · hypoxia · QSAR

## 1 Introduction

Hypoxia is a universal pathological process that accompanies a wide variety of pathologies. In general, hypoxia can be defined as a mismatch between the cell energy requirements and energy production in the mitochondrial oxidative phosphorylation system.

The causes of damaged energy production in a hypoxic cell can be respiratory disturbances, circulatory disorders in the lungs, disorders in blood oxygen-transport function, systemic, regional blood circulation and microcirculation disorders, endotoxemia. The immediate cause of this deficiency in the vast majority of pathological conditions is a decrease in the flow of oxygen into the mitochondria. As a result, inhibition of mitochondrial oxidation develops. Hypoxia also leads to an essential modification of the membrane enzymes.

Several approaches can be used to improve the energy status of the cell, one of which is the use of pharmacological preparations – antihypoxants.[1]

Currently, there are a number of synthetic antihypoxants on the market – trimetazidine, oliphene, ethylmethylhydroxypyridine succinate, and others.

A large number of cases of intolerance to synthetic drugs, side effects during their use, and sometimes the occurrence of drug-related complications due to their toxicity makes us pay attention to the possibility of using herbal preparations. Such an approach may allow finding new antihypoxic drugs that have low toxicity with activity close to the synthetic substances, and in some cases, even exceeding this activity.[2] Antihypoxic agents of synthetic origin are represented by the main five groups: fatty acid oxidation inhibitors, succinate-containing, and succinate-forming agents, natural components of the respiratory chain, artificial redox systems, macroergic compounds).[3] Unlike synthetic agents, the mechanisms of action for natural antihypoxants are poorly understood.

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Plant-based antihypoxants, which also often have an antioxidant and adaptogenic effects, are promising universal stress protectors. Their pharmacological effect is realized due to a combination of pleiotropic effects and can be used in various pathologies based on hypoxia, ischemia, and oxidative stress.[4]

The relationship between antihypoxic activity and the presence in the medicinal plant raw material of such groups of substances as anthracene derivatives, essential oils, saponins, coumarins, amino acids, flavonoids, phenolic alcohols, and various glycosides was shown.

Today, every second innovative active pharmaceutical ingredient is wholly or partly based on biologically active substances of plant origin. The demand for such preparations is traditionally high, and it is not decreasing. One of the objectives of modern medicine and biology is the search and creation of new drugs based on plant biomaterial and the use of plant objects as industrial producers of target metabolites.[5]

However, due to unfavorable environmental conditions, as well as an intensively rising level of consumption, there is a shortage of high quality natural medicinal raw materials. A new solution is the use of medicinal plant cell cultures as a renewable, environmentally friendly alternative source of biologically active compounds. Lines and strains of plant cell cultures producing target biologically active compounds can be the basis of industrial production of biomass and valuable secondary metabolites of plant origin for the creation of high-tech medicines intended for the prevention and correction of pathologies with high social significance.

The plant cell culture is a unique, experimentally created biological system – a population of plant somatic cells. It was shown that *in vitro* cells differ significantly from those of a whole plant in some characteristics. To a greater extent, this concerns the intensity of cell growth, as well as the features of the synthesis and accumulation of produced biologically active substances. Therefore, the study of the peculiarities of the metabolism of cell cultures of potentially medicinal species deserves special attention.

At present, more than a hundred of various plant cell cultures of medicinal plants producing biologically active substances have been obtained either at the level of the corresponding intact plant or in larger quantities. Among them – cell cultures of *Dioscorea deltoidea* and *Tribulus terrestris*, which tend to accumulate steroidal glycosides, *Panax japonicus* cell cultures, which synthesize triterpene glycosides (ginsenosides) in significant quantities.[6]

There are several studies on the antihypoxic properties of whole plants of *Dioscorea*, *Panax*, and *Tribulus ssp.* and their constituents. For instance, ginsenoside Rb1, which is the main ginsenoside that is extracted from the root of *Panax ginseng C.* A. Meyer has been reported to show various biological activities resisting ischemic injury in myocardial infarction, including prevention the apoptosis and death of hypoxic cardiomyocytes.[7] The decoction of *Dioscorea nipponica* could prolong the survival time of hypoxia under normal pressure in mice and has the anti-fatigue action.[8] A comparative study on the processes of oxygenation products indicates the presence of antihypoxic action in both cases in hematic hypoxia. Comparison of the effect of dry extract of aerial parts *Tribulus terrestris* in hematic, and histotoxic hypoxia indicates its selective effect on the blood oxygen-transporting function.[9]

The use of biologically active substances derived from plant cells and tissue cultures has shown their more pronounced efficiency in comparison with those obtained by traditional extraction methods from medicinal plant materials, which is mainly due to violations of the harvesting conditions and the inability to predict specific quantitative characteristics of the content of active ingredients in plant materials. Thus, it seems appropriate to evaluate the antihypoxic properties of a complex of biologically active substances accumulated by plant cell cultures of *Dioscorea deltoidea*, *Tribulus terrestris*, and *Panax japonicus*.

The computational evaluation of possible pharmacological effects of phytocomponents based on the analysis of structure-activity relationships is used to reduce the number of experiments. In this study, we used PASS software to predict biological activity spectra for structures of phytocomponents and PharmaExpert software for analysis of PASS prediction results to discover predicted pharmacological effects and mechanisms of action related to antihypoxic activity. The result of such investigation helped us to plan experimental studies of main phytocomponents of *Panax japonicus var. repens*, *Dioscorea deltoidea* and *Tribulus terrestris*. The workflow of the study is represented on Figure 1.

## 2 Materials and Methods

### 2.1 *In Silico* Tools

The structures of the main phytocomponents were found in PubChem Database and saved as MOL files.[10] Prediction of biological activity spectra was performed for the structures of phytocomponents by PASS (Prediction of Activity Spectra for Substances) software (version 2019). PASS is based on the use of MNA (Multilevel of Neighborhoods of Atoms) descriptors for the representation of structures of compounds and Bayesian-like algorithm for revealed structure-activity relationships.[11] The prediction of PASS is based on the data on more than 1 million structures of compounds and its biological activities. PASS predicts over 7000 types of biological activities (including pharmacotherapeutic effects, mechanisms of action, antitargets, toxic and side effects, change of gene expression, interaction with drug-metabolizing enzymes and transporters) with accuracy 95% calculated by leave-one-out cross-validation.[12] PASS prediction result for a structure is displayed as a list of names of biological activities with two values varied from 0 to 1: Pa – probability to be active and Pi – probability to be

  

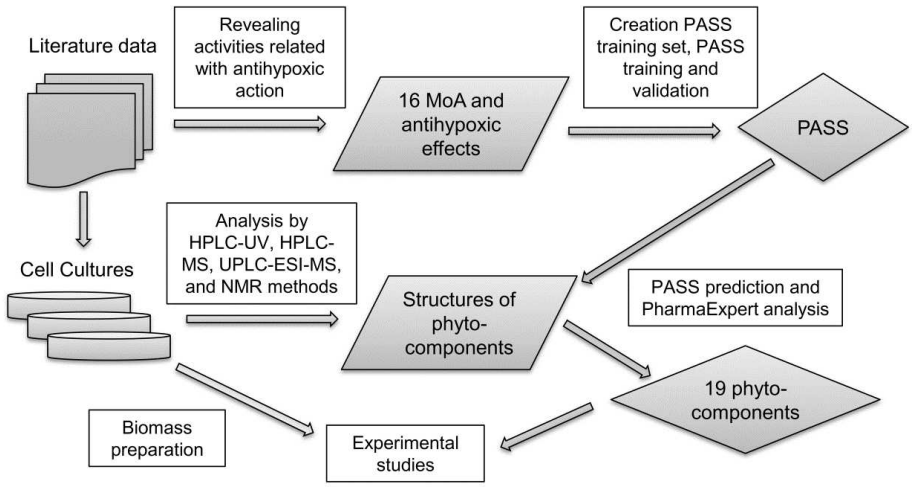


**Figure 1.** The workflow of the study. MoA – Mechanism of Action.

**Table 1.** The number of active compounds and accuracy of prediction of activities related to the antihypoxic effect.

| No | N | $AUC_{LOO\ CV}$ | $AUC_{10\text{-fold CV}}$ | Activity Type |
|---|---|---|---|---|
| 1 | 15 | 0.992 | 0.992 | 3-KAT inhibitor |
| 2 | 3646 | 0.928 | 0.927 | Antioxidant |
| 3 | 51 | 0.992 | 0.992 | Fatty acid oxidation inhibitor |
| 4 | 1322 | 0.939 | 0.937 | Free radical cavenger |
| 5 | 39 | 0.936 | 0.934 | Hexokinase 2 inhibitor |
| 6 | 26 | 0.931 | 0.911 | HIF-1 alpha stimulant |
| 7 | 70 | 0.920 | 0.919 | Antihypoxic |
| 8 | 328 | 0.990 | 0.990 | HIF prolyl hydroxylase inhibitor |
| 9 | 1514 | 0.929 | 0.926 | Antiischemic |
| 10 | 2274 | 0.938 | 0.937 | Ischemic stroke treatment |
| 11 | 19 | 0.951 | 0.946 | NQO1 stimulant |
| 12 | 15 | 0.999 | 0.999 | NQO1 modulator |
| 13 | 9 | 0.932 | 0.933 | Oxidative phosphorylation inhibitor |
| 14 | 15 | 0.999 | 0.999 | Potassium channel activator |
| 15 | 123 | 0.925 | 0.925 | Pyruvate kinase isozyme M2 stimulant |
| 16 | 6 | 0.920 | 0.913 | Succinate dehydrogenase inhibitor |

N – number of active compounds; $AUC_{LOO\ CV}$ – AUC value calculated by leave-one-out cross-validation procedure; $AUC_{10\text{-fold CV}}$ – AUC value calculated by 10-fold cross-validation procedure; 3-KAT – 3-ketoacyl-CoA thiolase; HIF – hypoxia inducible factor; NQO1 – NADPH Quinone Oxidoreductase 1.

inactive. Pa and Pi values are calculated independently. If Pa value is more than Pi it means that a compound has probability to be active.[11,12]

In this study, we created a particular training set, including the structure of approximately 23000 compounds tested on 16 effects and mechanisms of action related antihypoxic activity (Table 1). During the training of PASS, the accuracy of prediction for 16 activities was estimated using the leave-one-out (LOO CV) and 10-fold (10-fold CV) cross-validation procedures. The average accuracy of prediction (AUC – Area Under the Curve) of these activities was about 0.95 in both cases. This result reflects the robustness of the created SAR models.

PharmaExpert software[13] was used for the analysis of PASS prediction results to find relationships between predicted activities and select the more probable ones. PharmaExpert includes data on more than 15000 known activity-activity relationships. The combination of PASS and PharmaExpert was successfully used for the analysis of possible activities of phytocomponents and plant extracts.[13,14,15] PharmaExpert has also the possibility to reveal possible synergistic or additive pharmacological effects based on the analysis of PASS prediction results for phytocomponents of medicinal plants.

### 2.2 Plant Material

The following plant cell suspension cultures, deposited in the All-Russian Collection of Cell Cultures of Higher Plants

  

(Institute of Plant Physiology, Russian Academy of Sciences), were used:

- *Dioscorea deltoidea* Wall., strain IFR-DM-0.5-03 (super-producer of furostanol glycosides), collection-number 89;
- *Panax japonicus* var. *repens* (T.Nees) C.A.Mey, strain PJ-62, collection-number 62;
- *Tribulus terrestris* L, strain Tter8, collection-number 81.

Cultivation was carried out in flasks and in bioreactors (large-scale research facilities «All-Russian Collection of cell cultures of higher plants IPPRAS» and «Experimental biotechnological facility IPPRAS»).

The nutrient medium contained minerals, carbohydrates, vitamins and growth regulators according to collection passports. Bubble bioreactors of two types were used for biomass obtaining: (1) bubble nozzle conical fermenter (development by Institute of Plant Physiology RAS); the working volume of 15 L, point aerating device; (2) bubble apparatus (1T, OKBA, Yoshkar Ola); total volume of 630 L; the working volume of 550 L; aerating equipment of a ring-type.[16] The cells were collected at the end of the exponential phase of the growth cycle (12–16 days of cultivation). The cell mass was separated from the medium using a vacuum filter, washed with distilled water, and dried to constant weight at 55 –60 °C.

The biomass of used plant cell cultures was analyzed for the presence of secondary metabolites by HPLC-UV, HPLC-MS, UPLC-ESI-MS, and NMR methods. As a result, the following compounds were found in the biomass of used plant cell cultures:

1. *Panax japonicas* var. *repens* – Ginsenosides: Rg1, Malonyl-Rg1, Rb1, Malonyl-Rb1, Rb2, Malonyl-Rb2, Rc, Malonyl-Rc, Re, Rd, Malonyl-Rd, Rf, R0, Rh1, Rg2, Notoginsenoside R1, Chikusetsusaponin IVa, Zingibroside R1.[17,18]
2. *Dioscorea deltoidea* – 25(*S*)-Deltoside (25(*S*)-protodeltonin); Deltoside (protodeltonin); 25(*S*)-Protodioscin (protoneodioscin); Protodioscin.[19,20]
3. *Tribulus terrestris* – Pentahexosyl-hydroxy-diosgenin (furostanol type); Pentandroside F; 25(*R*)-pentandroside F; Hexosyl-25(*S*)-terrestrosin H; Hexosyl-25(*R*)-terrestrosin H; 25(*S*)-prototerrestrosin B; 25(*R*)-prototerrestrosin B; Terrestrinin B; 25(*R*)-terrestrinin B.[21]

Dried cell cultures biomass of *Panax japonicas* var. *repens*, *Dioscorea deltoidea*, and *Tribulus terrestris* were powdered and suspended in purified water at a ratio of 1:20 for pharmacological experiments. The most abundant compounds in cell cultures are considered as the main phytocomponents of plant preparations. The structures of the main phytocomponents are represented in Figures 2–4.

### 2.3 Animals

Outbred male mice (20 ± 2 g), kept under standard conditions were used in the experiments. The animals had free access to a standard pellet diet and water ad libitum. All the experimental procedures were conducted following the Bioethical Commission rules of the SPCPU (Protocol No. Mice-1/18 of 04.04.2018). When working with laboratory animals, the provisions of Good Laboratory Practice (GLP) for preclinical studies were respected.

### 2.4 Antihypoxic Activity

The cell cultures were administered intragastrically in the form of suspensions in purified water once 60 minutes before the start of hypoxia modeling. The control groups and three experimental groups for each model were used in the study: asphyctic hypoxia (normobaric hypoxia with hypercapnia), haemic hypoxia, and histotoxic hypoxia – each group comprised of 10 animals. Control groups were treated with purified water in equivalent volumes. Mexidol (Emoxypine succinate) was used as a reference preparation.

At the first stage, the effect of test substances in acute asphyctic hypoxia (AAH) as the most sensitive and universal model was evaluated. The study used dosages of test substances of 50 mg/kg, 100 mg/kg, 200 mg/kg and 300 mg/kg. According to the results of the experiment in the AAH model, the minimum effective dose for each substance was determined. At the second stage, the effect of substances in minimum effective dose was studied under conditions of acute haemic hypoxia (AHeH) and acute histotoxic hypoxia (AHtH).

*Acute asphyctic hypoxia (AAH).* Animals were placed individually in a tightly closed 200 ml glass container, and antihypoxic activity was estimated as survival time in minutes, which were converted to seconds for calculations.

*Acute haemic hypoxia (AHeH).* Animals were injected intraperitoneally with NaNO2 (300 mg/kg), in the form of a 10% solution in purified water, and antihypoxic activity was estimated as survival time in minutes, which were converted to seconds for calculations.

*Acute histotoxic hypoxia (AHtH)* was modeled by intraperitoneal administration of a 0.4% aqueous solution of sodium nitroprusside (20 mg/kg), which is an inhibitor of tissue respiration. Antihypoxic activity was estimated as survival time in minutes, which were converted to seconds for calculations.[22]

For all quantitative data, descriptive statistical methods were used. In essence, sample average values and standard error of the mean were calculated, according to recommendations of the Guidelines for the Preclinical Studies of Medicines.[23] Intergroup differences were analyzed by non-parametric methods for multiple comparisons – the Mann-Whitney test. Differences were determined at a 0.05 significance level. The software "Statistica 6.1" was used.

  

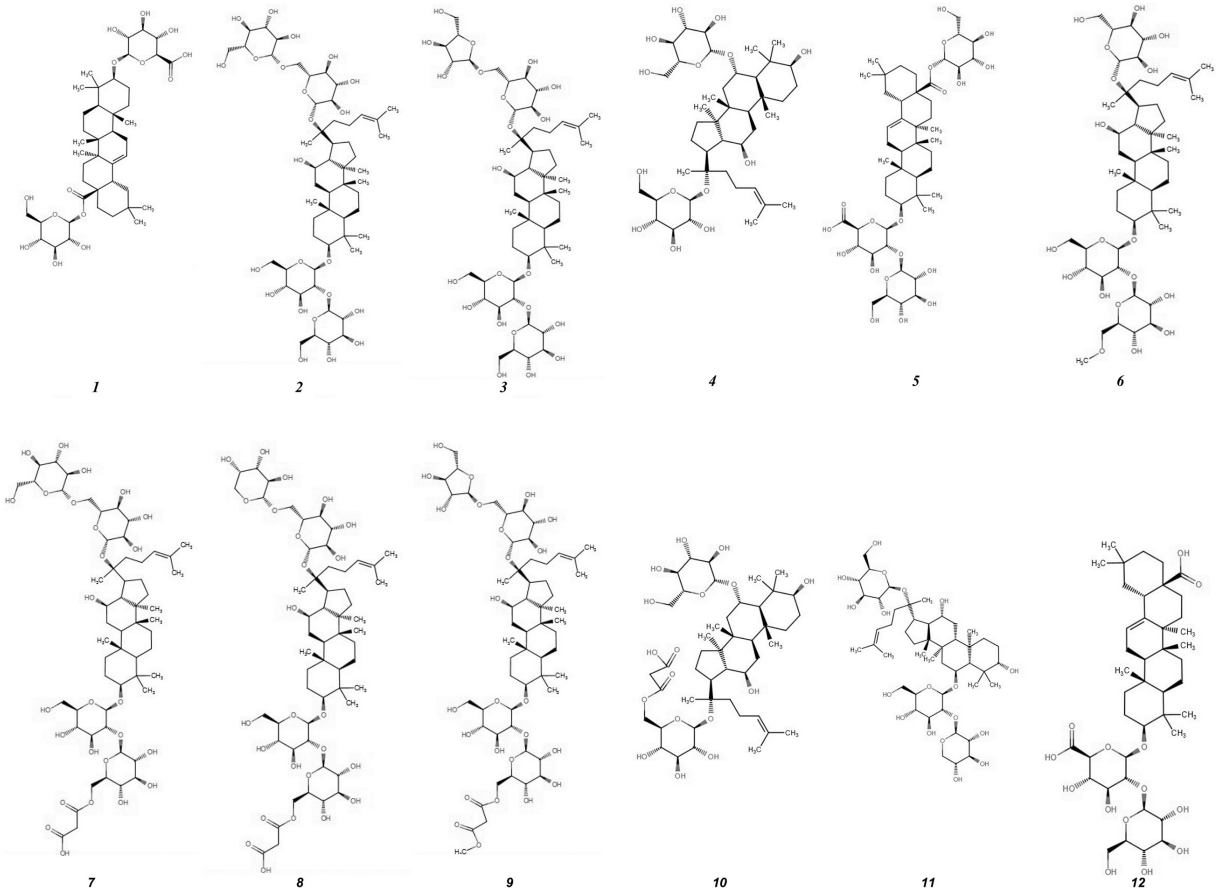


**Figure 2.** Structures of the main components of *Panax japonicus* var. *repens* cell culture. 1 – chikusetsusaponin IVa (1,8); 2 – ginsenoside Rb1 (1,8); 3 – ginsenoside Rc (1,0); 4 – ginsenoside Rg1 (5,3); 5 – ginsenoside R0 (40,7); 6 – malonyl-ginsenoside Rd (0,3); 7 – malonyl-ginsenoside Rb1 (22,6); 8 – malonyl-ginsenoside Rb2 (10,1); 9 – Malonyl-ginsenoside Rc (8,5); 10 – malonyl-ginsenoside Rg1 (2,6); 11 – notoginsenoside R1 (0,3); 12 – zingibroside R1 (1,2)

## 3 Results and Discussion

### 3.1 *In Silico* Evaluation of Biological Activity

PASS prediction was performed for the structures of the main components of the studied plants. Then PASS prediction results were analyzed by PharmaExpert for revealing activities related to antihypoxic activity. The results of PASS prediction are represented in Table 2.

Table 2 shows that only three from 16 PASS probable activities that were predicted for the studied phytocomponents with probability Pa more 0.3. Antioxidant activity was predicted with the highest probability for all phytocomponents. It is varied from 0.757 (Pentandroside F) to 0.948 (Notoginsenoside R1).

Free radical scavenger activity was predicted for eight compounds. Pa value for this activity varies from 0.411 (Zingibroside R1) to 0.613 (Prototerrestrosin B). Antihypoxic activity was predicted with Pa value between 0.303 (Malonyl-ginsenoside Rc) and 0.378 (Zingibroside R1) for 11 compounds. PASS prediction results in Table 2 for *Dioscorea deltoidea* (Protoneodioscin, Protodioscin, Protodeltonin) are the same.

The result of the prediction mentioned in Table 2 shows that the most of the main phytocomponents may reveal antioxidant, antihypoxic, and free radical scavenger effects leading to additive or synergistic action. It is a reason to study the antihypoxic effect of cell cultures of *Dioscorea deltoidea*, *Panax japonicus*, *and Tribulus terrestris* in the experiment.

To search additional therapeutic activities related to the antihypoxic effect for the studied phytocomponents, we used the current version of PASS (version 2019). PASS prediction was made for the structures of the main components of the studied plants. Then PASS prediction results were analyzed by PharmaExpert for revealing activities related to antihypoxic activity. The results of PASS prediction and PharmaExpert analysis are represented in Table S1. The numbers of active compounds and accuracy of PASS prediction for activities from Table S1 are presented in Supplement (Table S2).

  

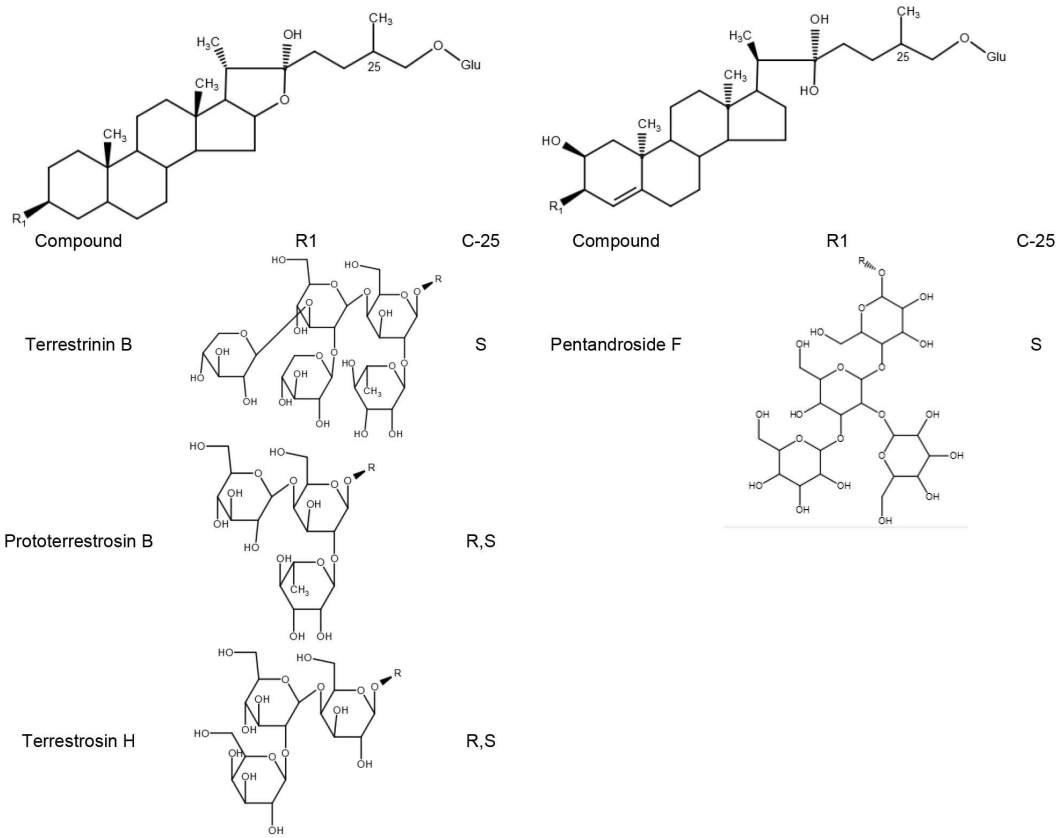


**Figure 3.** Structures of the main components of *Tribulus terrestris* cell culture.

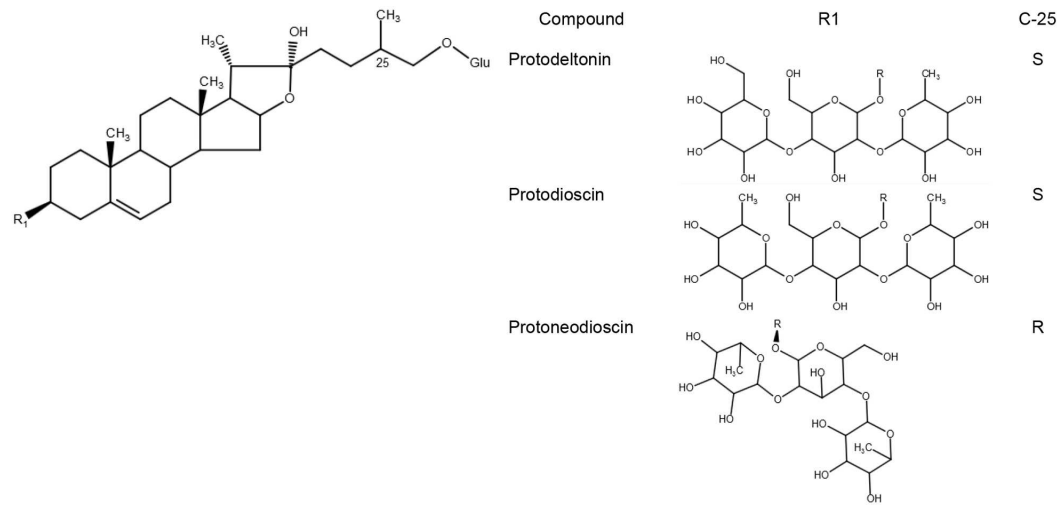


**Figure 4.** Structures of the main components of *Dioscorea deltoidea* cell culture.

Table S1 shows that neuroprotective activity was predicted for all compounds with high probability. Cholesterol antagonism related to neuroprotective activity and apoptosis antagonism associated with cytoprotection were also predicted with a high probability for most of the compounds. Antagonism to glycine receptor and agonism to nerve growth factor related to neuroprotective activity were predicted for some compounds and may increase their therapeutic effects.

  

**Table 2.** The most probable pharmacological effects and mechanisms of action related to antihypoxic activity, which were predicted by PASS.

| ID | Compound | Pa | Pi | Activity |
|---|---|---|---|---|
| *Dioscorea deltoidea* | | | | |
| 1 | Protoneodioscin | 0.918 | 0.004 | Antioxidant |
| 2 | Protodioscin | 0.540 | 0.033 | Free radical scavenger |
| 3 | Protodeltonin | | | |
| *Panax japonicus* | | | | |
| 1 | Chikusetsusaponin IVa | 0.842 | 0.005 | Antioxidant |
| | | 0.452 | 0.048 | Free radical scavenger |
| | | 0.338 | 0.005 | Antihypoxic |
| 2 | Ginsenoside Rb1 | 0.958 | 0.004 | Antioxidant |
| | | 0.334 | 0.005 | Antihypoxic |
| 3 | Ginsenoside Rc | 0.958 | 0.004 | Antioxidant |
| | | 0.334 | 0.005 | Antihypoxic |
| 4 | Ginsenoside Rg1 | 0.943 | 0.004 | Antioxidant |
| 5 | Ginsenoside Ro | 0.858 | 0.005 | Antioxidant |
| | | 0.459 | 0.047 | Free radical scavenger |
| | | 0.356 | 0.005 | Antihypoxic |
| 6 | Malonyl ginsenoside Rd | 0.909 | 0.004 | Antioxidant |
| | | 0.319 | 0.006 | Antihypoxic |
| 7 | Malonyl-ginseniside Rb1 | 0.917 | 0.004 | Antioxidant |
| | | 0.300 | 0.008 | Antihypoxic |
| 8 | Malonyl-ginseniside Rb2 | 0.914 | 0.004 | Antioxidant |
| | | 0.352 | 0.005 | Antihypoxic |
| 9 | Malonyl-ginsenoside Rc | 0.911 | 0.004 | Antioxidant |
| | | 0.303 | 0.008 | Antihypoxic |
| 10 | Malonyl-ginsenoside Rg 1 | 0.902 | 0.005 | Antioxidant |
| 11 | Notoginsenoside R1 | 0.948 | 0.004 | Antioxidant |
| | | 0.362 | 0.005 | Antihypoxic |
| 12 | Zingibroside R1 | 0.829 | 0.005 | Antioxidant |
| | | 0.378 | 0.005 | Antihypoxic |
| | | 0.411 | 0.057 | Free radical scavenger |
| *Tribulus terrestris* | | | | |
| 1 | Pentandroside F | 0.757 | 0.008 | Antioxidant |
| | | 0.409 | 0.058 | Free radical scavenger |
| 2 | Terrestrinin B | 0.939 | 0.004 | Antioxidant |
| | | 0.548 | 0.032 | Free radical scavenger |
| 3 | Prototerrestrosin B | 0.805 | 0.004 | Antioxidant |
| | | 0.613 | 0.005 | Free radical scavenger |
| 4 | Terrestrosin H | 0.931 | 0.004 | Antioxidant |
| | | 0.527 | 0.035 | Free radical scavenger |

Pa – probability to be active; Pi – probability to be inactive.

These activities, together with antioxidative effect and inhibition of lipid peroxidase, may increase of action each other and increase of antihypoxic effect of compounds. The antiinflammatory effect and its mechanisms of actions (Interleukin 10 agonist and Transcription factor NF kappa B inhibitor) were also predicted with high probability. They may be related to the therapeutic antihypoxic effects of the studied plants.

To reveal possible synergistic or additive effects, we used drug-drug interaction analysis implemented in PharmaExpert.[14] Such analysis is based on predicted biological activity spectra for individual compounds and the knowledge about mechanism-effect relationships. We consider that if either a particular mechanism of action or a few mechanisms of action causing the same pharmacotherapeutic effect were predicted for several compounds, then such interaction could lead to additive/synergistic activity.[14] Table 3 shows possible synergistic or additive effects related to the antihypoxic action of the studied main phytocomponents. All activities represented in Table 3 were predicted with probability Pa more 0.5.

Table 3 shows that the main phytocomponents may reveal several possible synergistic or additive effects related to antihypoxic activity. Therefore the activity of extracts of the studied plants may be more active in comparison with the value of the activity of individual main phytocomponents. *Dioscorea deltoidea* is not represented in Table 3 because its phytocomponents different only stereochemically and have the same PASS prediction result.

  

**Table 3.** Possible synergistic or additive effects of main phytocomponents and related activities predicted by PASS with probability Pa more 0.5.

| Possible synergic or additive effect | Predicted activities related with possible synergic or additive effect (IDs of phytocomponents in Table 2) |
|---|---|
| *Panax japonicus* | |
| Neuroprotector | Apoptosis antagonist (1–12); Chloride channel blocker (8, 10); Cholesterol antagonist (1–12); Free radical scavenger (1, 5, 6, 9, 11, 12); Glycine receptor antagonist (1–6, 9, 11, 12); Hypocalcaemic (1–6, 9, 11); Immunosuppressant (1–12); Lipid peroxidase inhibitor (1, 3–12); Nerve growth factor agonist (2–6, 9, 11, 12); Neuroprotector (1–12); Nitric oxide scavenger (2–4, 6, 9, 11) |
| Antithrombotic | Antithrombotic (1–12); Transcription factor NF kappa B inhibitor (1–12) |
| Atherosclerosis treatment | Antioxidant (1–12); Atherosclerosis treatment (5, 7, 8, 10, 11, 12); Cholesterol antagonist (1–12); Cholesterol synthesis inhibitor (3); Protein-tyrosine phosphatase inhibitor (7, 8, 10); Transcription factor NF kappa B inhibitor (1–12) |
| Antiinflammatory | Anaphylatoxin receptor antagonist (7, 8, 10); Antiinflammatory (1–12); Free radical scavenger (1, 5, 6, 9, 11, 12); Hyaluronic acid agonist (7, 8, 10); Immunosuppressant (1–12); Non-steroidal antiinflammatory agent (7, 8, 10); Phospholipase A1 inhibitor (2–4, 6, 9, 11, 12); Protein-tyrosine phosphatase inhibitor (7, 8, 10); Transcription factor NF kappa B inhibitor (1–12) |
| Antioxidant | Antioxidant (1–12); Lipid peroxidase inhibitor (1, 3–12) |
| Vasodilator, coronary | Vasodilator, coronary (7, 8, 10) |
| *Tribulus terrestris* | |
| Neuroprotector | Apoptosis antagonist (1–4); Chloride channel blocker (1, 2); Cholesterol antagonist (1–4); Free radical scavenger (3); Immunosuppressant (1–4); Membrane permeability enhancer (1, 2); Nerve growth factor agonist (1–4); Neuroprotector (1–4) |
| Atherosclerosis treatment | Antioxidant (3, 4); Atherosclerosis treatment (1, 2); Cholesterol antagonist (1–4); Cholesterol synthesis inhibitor (4); Transcription factor NF kappa B inhibitor (1–4) |
| Respiratory analeptic | Respiratory analeptic (1–4) |
| Antiinflammatory | Antiinflammatory (1–4); Free radical scavenger (3); Hyaluronic acid agonist (1, 2); Immunosuppressant (1–4); Interleukin 10 agonist (1–3); Membrane permeability enhancer (1, 2); Phospholipase A1 inhibitor (1–4); Transcription factor NF kappa B inhibitor (1–4) |
| Antithrombotic | Antithrombotic (1–4); Transcription factor NF kappa B inhibitor (1–4) |

We also performed the literature search to analyse which predicted biological activities correspond to the experimental ones. The results are shown in Table 4.

The comparison of the prediction results given in Table S1 and the literature data presented in Table 4 shows that they coincide. The comparison, along with the obtained experimental results, indicates the advisability of using the computational tools described in the article to study the antihypoxic potential of medicinal plants and their phytocomponents.

### 3.3 Experimental Results

In the AAH model, the cell cultures biomass of *Dioscorea deltoidea* exhibits antihypoxic activity with a statistically significant difference from the control group ($p < 0.05$) at a dose of 100 mg/kg. This dose was selected for further studies on other acute hypoxia models.

The cell cultures biomass of *Panax japonicus* showed weak antihypoxic activity at a dose of 100 mg/kg in the form of an aqueous suspension; the ethanolic suspension and the extract obtained using ultrasound exhibit a pro-hypoxic effect. For further studies, an aqueous suspension of cell cultures biomass at a dose of 100 mg/kg was selected.

The cell cultures biomass of *Tribulus terrestris* exhibits antihypoxic activity in the AHH model at doses of 100 mg/kg and 200 mg/kg. For further studies, a minimum effective dose of 100 mg/kg was chosen.

As shown in Table 5, the treatment with the cell culture preparations of Dioscorea deltoidea (100 mg/kg), *Panax japonicus* (100 mg/kg), and *Tribulus terrestris* (100 mg/kg) significantly prolonged the survival time, compared to the control group ($p < 0.05$) in AHtH model. Wherein the effect of *Panax* and *Tribulus* preparations exceeded the activity of the reference preparation.

In the AHeH model all the preparations moderately prolonged survival time compared to the control group. Antihypoxic activity was especially pronounced for *Dioscorea deltoidea*.

## 3 Conclusions

Computational estimation of pharmacological potential for separate phytoconstituents and their mixtures allows to (a) select medicinal plants with the desirable effects, (b) detect the impact of particular phytoconstituents, and (c) shed light onto molecular mechanism(s) of the observed actions. The prediction of biological activities spectra for the main phytocomponents from plant cell cultures of three species

  

**Table 4.** Predicted effects of the phytocomponents that are confirmed by the published data.

| Phytocomponent | Action |
|---|---|
| *Panax japonicus var. repens* | |
| Ginsenoside Rg1 | Nootropic[24] |
| | Neuroprotective[25] |
| Ginsenoside Rb1 | Nootropic[24] |
| | Reduce liver triglyceride level[26] |
| | Protective on myocardiac ischemic and reperfusion injuries[27] |
| | Neuroprotective[24] |
| Ginsenoside Rb2 | Stimulate epidermal cell proliferation[28] |
| | Protective effects on myocardiac ischemic and reperfusion injuries[27] |
| | Neuroprotective[29] |
| Ginsenoside Rc | Antioxidant[30] |
| | Anti-inflammatory[31] |
| Ginsenoside Re | Antioxidant, protecting cardiomyocytes from oxidant injury[32] |
| | Protective effect against cerebral ischemia –reperfusion injury[33] |
| Ginsenoside Rd | Neuroprotective[34] |
| Ginsenoside Rf | Increase basal levels of hepatic apolipoprotein (apo) AI and C–III mRNA[35] |
| Ginsenoside R0 | Neuroprotective[36] |
| Ginsenoside Rh1 | Antiallergic and anti-inflammatory[37] |
| Ginsenoside Rg2 | Antioxidant[38] |
| Notoginsenoside R1 | Cardioprotective[39] |
| | Reduces renal injury and dysfunction by attenuation of apoptosis and inflammation[40] |
| | Attenuates microcirculatory disturbance induced by lipopolysaccharide[41] |
| | Anti-inflammatory[42] |
| Chikusetsusaponin Iva | Antitrombotic[43] |
| | Anti-inflammatory[44] |
| *Dioscorea Deltoidea* | |
| Protodioscin | Neuroprotective, anti-inflammatory, anti-apoptosis[45] |
| | Anti-hyperlipidemic[46] |

**Table 5.** Effects of the cell cultures biomass on survival time in AAH, AHtH, and AHeH models.

| Groups | Dose (mg/kg) | Hypoxia model AAH[a], survival time, s | AHtH[b], survival time, s | AHeH[c], survival time, s |
|---|---|---|---|---|
| *Dioscorea deltoidea* | 50 | 1925 ± 596 | – | – |
| | 100 | 2966 ± 1565 | 754 ± 71* | 738 ± 237* |
| | 200 | 2062 ± 547 | – | – |
| | 300 | 2312 ± 504 | – | – |
| *Panax japonicus* susp. in water | 25 | 1504 ± 162 | – | – |
| | 100 | 1727 ± 287 | 920 ± 253* | 609 ± 55* |
| *P. japonicus* susp. in ethanol | 100 | 1208 ± 162* | – | – |
| *P. japonicus* susp. in water + ultrasound | 100 | 1383 ± 131* | – | – |
| *Tribulus terrestris* | 100 | 2212 ± 676 | 797 ± 57* | 644 ± 120 |
| | 200 | 2722 ± 920 | – | – |
| Mexidol | 100 | 3382 ± 542* | 635 ± 31* | 672 ± 42* |
| Control | – | 1649 ± 181* | 434 ± 41* | 492 ± 64* |

[a] AAH – Acute asphyctic hypoxia.
[b] AHtH – Acute histotoxic hypoxia.
[c] AHeH – Acute haemic hypoxia.
* Statistically significant result.

and the analysis of the predicted results showed perspectives of experimental study of antihypoxic effect for the appropriate cell culture. The experimental study confirmed the antihypoxic activity of the studied plant cell cultures. Antihypoxic activity was determined for *Dioscorea*, *Panax*, and *Tribulus* extracts containing the sum of natural products. Therefore, at this stage, any assumptions about the relationship of biological activity with structural features seem premature. Undoubtedly, understanding the structural patterns responsible for a certain activity is a very

  

important step in establishing the mechanism of the pharmacological activity of natural products. Future work on the isolation and pharmacological study of individual compounds will allow us to draw such conclusions more reasonably.

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## *Conflict of Interest*

None declared.

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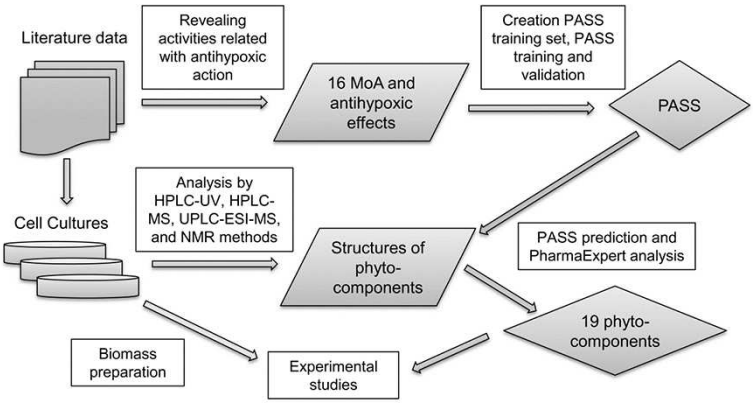


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**Antihypoxic Action of *Panax Japonicus*, *Tribulus Terrestris* and *Dioscorea Deltoidea* Cell Cultures: In Silico and Animal Studies**