# Study of the phenolic composition and antioxidant activity of the adaptogenic agent "Adaptofit" in vitro and in vivo

P.B. Lubsandorzhieva[one], M.V. Baldandorzhieva[one], L.N. Shantanova[one], L.V. Osadchuk[2]
([one]FSBSI Institute of General and Experimental Biology SB RAS, Ulan-Ude, [2]FGBUN Institute cytology and genetics SB RAS, Novosibirsk)

The study of phenolic composition and antioxidant activity of adaptogenic remedy "Adaptophyte" in vitro and in vivo

PB Lubsandorzhieva[one], MV Baldandorzhieva[one], LN Shantanova1, LV Osadchuk[2] [one]The Institute of General and Experimental Biology of SB RAS (Ulan-Ude, Russia)
[2]The Institute of Cytology and Genetics of SB RAS (Novosibirsk, Russia)

SUMMARY

The quantitative content of the main groups of biologically active substances and the antioxidant activity in vitro and in vivo of the 15-component adaptogenic agent "Adaptofit" were determined. For in vitro experiments, the ethyl acetate fraction, decoction and dry extract from Adaptophyt were used. In the dry extract from "Adaptofit", the HPLC method established the content of the following phenolic compounds: gallic (31.7%), chlorogenic (21.8%), isoferulic (8%), chicoric (7.4%) acids, vitexin (3, 3%), routine (2.6%). The data of experiments in vitro showed that the most effective Fe2 + - chelating activity is the dry extract, superoxide radical-binding activity - ethyl acetate fraction and dry extract, NO-binding activity - ethyl acetate fraction. Moreover, the ethyl acetate fraction most effectively suppresses the formation of malondialdehyde in the model with lipoproteins than a dry extract or a decoction of Adaptofit. It was shown that "Adaptofit" inhibits the rate of formation of free radical oxidation products and activates the endogenous antioxidant system in Wistar rats against the background of intense physical exertion.

Key words: adaptogenic agent, phenolic compounds, antioxidant activity in vitro and in vivo.

RESUME

The content of biologically active compounds and the antioxidant activity in vitro and in vivo in a 15-component adaptogenic herbal remedy “Adaptophyte” were studied. The ethyl acetate fraction, decoction and dry extract of “Adaptophyte” were used for in vitro study. The content of following phenolic substances in the dry extract of “Adaptophyte” was established by the HPLC method: gallic (31.7%), chlorogenic (21.8%), izoferuloic (8%), chicoric (7.4%) acids, vitexin (3.3%) and rutin (2.6%). The in vitro experimental data showed that the most effective Fe 2 + - chelating activity possessed the dry extract, the strong superoxide radical scavenging activity possessed the ethyl acetate fraction and dry extract, and the - NO scavenging activity - ethyl acetate fraction. In addition the ethyl acetate fraction suppressed the formation of malone dialdehyde more effectively than the dry extract or decoction in the lipoprotein model. It has been shown that “Adaptophyte” inhibited the intensive formation of free radical oxidation products and activated the endogenic antioxidant system in rats of the strain Wistar under intensive physical exercises.

Keywords: adaptogenic remedy, phenolic compounds, antioxidant activity in vitro and in vivo.

## INTRODUCTION

Free radical oxidation (FRO) of macromolecules plays a key role in the pathogenesis of various chronic diseases leading to the depletion of the body's defenses. It is known that phenolic compounds (PCs) of phytopreparations, depending on the structure, lipophilicity / hydrophobicity and concentration, have antioxidant activity [1]. Experimental data on the antioxidant activity (AOA) of phytopreparations obtainedin vitro, are not always confirmed in vivo due to the metabolism of PS in the gastrointestinal tract (GIT) [2]. Previously, we developed a formulation of a 15-component adaptogenic agent "Adaptofit" (conventional name), and using the HPLC method studied its flavonoid composition in the ethyl acetate fraction, where rutin, quercetin, luteolin, isorhamnetin, isoquercitrin, narcissin, kaempferol, isoquercitrin -Orutinosyl rhamnoside, cynaroside, isoscutellarein-7-O-β-xylopyranoside [3].

The purpose of this work is to study the quantitative content of the main groups of biologically active substances (BAS), phenolic composition by HPLC, and antioxidant activity (AOA) of the adaptogenic agent "Adaptofit" in vitro and in vivo.

MATERIALS AND METHODS

The adaptogenic remedy "Adaptofit" contains: rhizomes and roots of safflower Leuzea Rhaponticum carthamoides (Willd) DC. (13 hours), elecampane high Inula helenium L. (7 hours), marshmallow Althaea officinalis L. (5 hours), marsh calamus Acorus calamus L. (5 hours), ginger Zingiber officinale Roscoe (4.5 hours), inflorescences of calendula officinalis Calendula officinalis L. (13 hours), fruits of cardamom Elettaria cardamomum Maton. (9 hours), nutmeg Myristica fragrans Houtt. (5 hours), pomegranate Punica granatum L. (11.5 hours), long pepper Piper longum L. 6.5 hours), juniper Juniperus communis L. 5 hours), bark of the cinnamon tree Cinnamomum cassia (4.5 hours), grass Knotweed Polygonum aviculare L. (5 hours), black leaves of bergenia thick-leaved Bergenia crassifolia (L.) Fritsch. (3 hours) and chitosan (3 hours). Pharmacopoeial raw materials,

- in the trading network, black (overwintered) leaves of B. crassifolia were collected on the Ulan-Burgasy ridge in the Baikal region of Buryatia in the autumn-spring periods of 2010-2011.

To obtain a dry extract, 30 g (accurately weighed) of "Adaptofit" was extracted sequentially with 80% and 40% ethyl alcohol, hot water twice, 150 ml each, the extraction was filtered, the alcohol was removed, the aqueous residue was dried in a vacuum drying oven. The yield of the dry extract was 38.7% of the mass of the initial mixture. To obtain an ethyl acetate fraction, 5 g (accurately weighed) of "Adaptofit" was treated twice with 25 ml of hexane, then with 25 ml of chloroform (3-fold extraction) to remove lipophilic substances. The meal was successively extracted with 80% and 40% ethyl alcohol, hot water three times 50 ml each, the extracts were filtered off, the alcohol was removed, the aqueous residue was concentrated to 10–15 ml. The aqueous residue was treated with 30 ml of ethyl acetate (5 times), the extract was separated from the aqueous layer, the solvent was removed, the dry residue of the ethyl acetate fraction was dissolved in 25 ml of 96% ethyl alcohol. Decoction (1:10) "Adaptofit" for experimentsin vitro prepared according to pharmacopoeial method, for experiments in vivo, a suspension of decoction with meal was used (the degree of grinding initial mixture 0.18 mm) due to the insolubility of chitosan in water. The quantitative content of biologically active substances in Adaptofit was determined by known methods: tannins, flavonoids, water-soluble polysaccharides, essential oils - pharmacopoeial, carotenoids [4] and triterpene saponins [5] - spectrophotometric, amino acids - using a reaction with 1% alcohol solution of ninhydrin. The study of the quantitative composition of individual substances in the dry extract of Adaptofit was carried out on a high-performance liquid chromatograph (GILSTON), model 305 (France); manual injector, model RHEODYNE 7125 (USA) with subsequent computer processing of research results using the Multichrom program for Windows. A mixture of methanol - water - concentrated phosphoric acid in a ratio of 400: 600: 5 was used as a mobile phase. Detection was carried out using a GILSTON UV / VIS UV detector at a wavelength of 254 nm. Standard samples of gallic, chlorogenic, isoferulic, chicoric acids, vitexin, and rutin were prepared in 70% ethanol at a concentration of 0.05%. The quantitative ratio of substances in the analyzed solution was established by the method of normalization by the area of chromatographic peaks.

To establish the spectrum of AOA water extraction (1:10), dry extract, ethyl acetate fraction "Adaptofit", the nitroprusside method was used to determine the NO-binding activity [6]; phenanthroline method - for $Fe^{2+}$-chelating activity [7]. The determination of superoxide anion-radical-binding activity was carried out according to the method [8]; and for the determination of TBK-active products, the method with yolk lipoproteins (YLL) was used [9].

Experiments in vivo performed on 28 white rats line Wistar of both sexes 180–220 The animals were kept in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. To study the effect of "Adaptofit" on the intensity of the SRO process, a model of animals swimming in a pool with a weight of 7% of body weight was used. The rats of the experimental group were intragastrically injected with a decoction (1:10) of Adaptophyt (suspension containing decoction and meal) in a volume of 10 ml / kg for 7 days before stress exposure (once a day, 30 minutes before feeding). Animals of the control group received an equivalent volume of distilled water according to a similar scheme. A dealcoholized extract of liquid Eleutherococcus in a volume of 5 ml / kg was used as a reference drug. After 7

days from the beginning of drug administration, the intensity of FRO processes was assessed by the content of catalase [10], reduced glutathione [11], and malondialdehyde (MDA) in blood serum [12]. Experimental data are shown in Table 4.

To process the results, the Advanced Grapher 2.0 and Statistica 6.0 programs were used.

## RESULTS AND ITS DISCUSSION

In the initial mixture of "Adaptofit" and dry extract, the content of the main groups of biologically active substances was determined: essential oils, carotenoids, triterpene saponins, tannins, flavonoids, amino acids, water-soluble polysaccharides (Table 1). From the initial mixture into the dry extract "Adaptofit" containing lipophilic (extraction with 80% ethyl alcohol), medium polar (extraction with 40% ethyl alcohol) and water-soluble (water extraction) substances, most of the BAS was extracted, except for volatile substances (Table 1). The presence of phenolic acids was detected by HPLC in the dry extract of Adaptofit: gallic, chlorogenic, isoferulic, chicory; flavonoids: vitexin and rutin (Table 2). It should be noted that gallic acid is the dominant substance in the Adaptophyt phenolic complex.

Table 1

Content of biologically active substances in "Adaptofit" and dry extract from it

| Наименование | Содержание | |
|---|---|---|
| | «Адаптофит» | Экстракт сухой «Адаптофита» |
| Эфирные масла, % | 1,7 | - |
| Каротиноиды в пересчете на β-каротин, мг% | 1,2 | 0,6 |
| Тритерпеновые сапонины, в пересчете на олеаноловую к-ту, % | 1,4 | 3,4 |
| Дубильные вещества, в пересчете на таннин, % | 1,3 | 6,5 |
| Флавоноиды, в пересчете на рутин, % | 0,6 | 1,4 |
| Свободные аминокислоты, в пересчете на глютаминовую к-ту, % | 0,3 | 0,8 |
| Водорастворимые полисахариды, % | 18,2 | 25,0 |

Примечание: данные представляют среднее из трех определений; прочерк означает, что вещества не обнаружены.

table 2

The quantitative content of some phenolic compounds in dry extract "Adaptofit"

(HPLC method)

| Наименование | Время удерживания, мин | Содержание, % |
|---|---|---|
| Галловая кислота | 3,3 | 31,7 |
| Хлорогеновая кислота | 3,8 | 21,8 |
| Изоферуловая кислота | 5,5 | 8,0 |
| Цикориевая кислота | 6,8 | 7,4 |
| Витексин | 10,3 | 3,3 |
| Рутин | 15,5 | 2,6 |

Experiments in vitro, according to the determination of AOA extracts from Adaptofit, showed that water-soluble substances with volatile components (decoction), the total extraction of biologically active substances of different polarity (dry extract), the fraction of phenolic, predominantly flavonoid substances (ethyl acetate fraction) have antiradical activity in relation to activated forms of oxygen: $O_2$ and NO, and also exhibit $Fe^{2+}$ -chelating activity in vitro (tab. 3). Thus, the water-soluble substances of "Adaptofit" exhibit moderate $Fe^{2+}$-chelating activity ($IC_{50}$= 128 μg / ml) compared to dry extract ($IC_{50}$= 24 μg / ml) containing both hydrophilic and lipophilic substances. Inactivating ability against $O_2$ - radicals in the extracts of "Adaptofit" decreases in the order: ethyl acetate fraction> dry extract> water extract (Table 3). The ethyl acetate fraction containing flavonoid compounds [3] also effectively exhibits NO-binding activity at the lowest concentration - $IC_{50}$ = 239 μg / ml, which is 4.5 times higher than the activity of ascorbic acid ($IC_{50}$ = 1140 μg / ml). In addition, the ethyl acetate fraction most effectively prevents the peroxidation of the biological substrate in the VLT model, suppressing the formation of MDA in

lowest concentration ($IC_{50}$ = 33 μg / ml), which exceeds the activity of the reference substance - ascorbic acid ($IC_{50}$ = 50 μg / ml) in this test system (Table 3).

Thus, the most pronounced antioxidant activity in various models in vitro have an ethyl acetate fraction containing flavonoids [3], as well as a dry extract containing flavonoids, phenolic acids (Table 2), which confirms the predominant contribution of PS to the total AOA "Adaptofit".

Experimental data in vitro confirmed by experimental data on the effect of "Adaptofit" on the intensity of the FRO processes and the state of the antioxidant system of the organism of white rats against the background of intense physical activity (Table 4). In animals, against the background of maximum physical activity, there is a significant increase in the level of MDA in the blood, as well as a decrease in the activity of catalase and SOD of blood serum, which indicates the induction of lipid peroxidation and inhibition of the activity of the endogenous antioxidant system of the body as a result of swimming until complete fatigue. Against the background of the preventive introduction of "Adaptofit" in the indicated dose, less pronounced changes in the indices of free radical oxidation and parameters of the antioxidant system are noted. Thus, the concentration of MDA in the blood serum of the animals of the experimental group was 38% less, of the eleutherococcus extract - by 37%, compared with the same indicators in the rats of the control group.

Table 3

Antioxidant activity of "Adaptofit" extracts in vitro

| Наименование | Концентрация ($IC_{50}$), мкг/мл | | | |
|---|---|---|---|---|
| | ЖЛП-метод | $Fe^{2+}$ | $O_2^{\cdot-}$ | NO |
| «Адаптофит» - отвар (1:10) | 312 ± 12 | 128 ± 10 | 370 ± 12 | 2825 ± 45 |
| «Адаптофит» - этилацетатная фракция | 33 ± 1 | - | 11 ± 1 | 239 ± 11 |
| «Адаптофит» - экстракт сухой | 270 ± 3 | 24 ± 1 | 18 ± 1 | > 5000 |
| Аскорбиновая кислота [a] | 50 ± 1 | 0.15 ± 0.01 | 101 ± 3 | 1140 ± 34 |

Примечание: ЖЛП-метод – антиоксидантная активность в отношении накопления ТБК-активных продуктов, $Fe^{2+}$ – $Fe^{2+}$-хелатирующая активность, $O_2^{\cdot-}$ – связывание супероксид-анион радикала, NO – связывание молекул оксида азота; [a] вещество сравнения, прочерк означает отсутствие данных.

Table 4

Influence of "Adaptofit" on the intensity of free radical oxidation processes and the state antioxidant system in rat strain Wistar during intense physical activity

| Показатель | Группы животных | | |
|---|---|---|---|
| | Контроль (n=8) | Элеутерококк (n=10) | «Адаптофит» (n=10) |
| МДА, нмоль/мл | 6,3 ± 0,3 | 4,0 ± 0,3 | 3,9 ± 0,2 |
| Каталаза, мкат/л | 13,7 ± 0,3 | 15,2 ± 0,3 | 15,7 ± 1,3 |
| Восстановленный глутатион, ммоль/л | 6,4 ± 0,5 | 9,0 ± 0,1 | 8,2 ± 0,3 |

Примечание: МДА – малоновый диальдегид; n – количество животных в группе.

Thus, the results of the studies carried out indicate that the adaptogenic agent "Adaptofit" exhibits antioxidant activity as in vitro and in vivo.

CONCLUSIONS

It was found that "Adaptofit" contains 1.7% essential oil, 1.3% tannins, 0.6% flavonoids, 1.2% carotenoids, 1.4% triterpene saponins, 0.3% amino acids, 18.2% water-soluble polysaccharides. The dry extract of "Adaptofit" contains gallic (31.7%), chlorogenic (21.8%), isoferulic (8.0%), chicory (7.4%) acids, as well as vitexin (3.3% ) and routine (2.6%).

The decoction, dry extract and ethyl acetate fraction of "Adaptofit" have radical-binding, metal-chelating activity, as well as the ability to inhibit lipid peroxidation. Most effective antioxidant activity in vitro, the ethyl acetate fraction is characterized.

In experiments on Wistar rats, which were subjected to intense physical exertion, it was shown that Adaptofit inhibits the rate of formation of free radical oxidation products and activates the endogenous antioxidant system.

This work was carried out with the financial support of the Presidium of the SB RAS (Integration project no. 57).

LITERATURE

1. Critical reviews of oxidative stress and aging. Advances in Basic Science, Diagnostics and Intervention // Ed. RG Cutler, H. Rodriquez. –New Jersey, London, Singapore, Hong Kong: World scientific. - 2003. - VI - 822 p.

2. Houghton PJ, Howes M.-J., Lee CC et al. Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant // Journal of Ethnopharmacology. - 2007. - V. 110. - P. 391–400.

3. Lubsandorzhieva PB, Boldanova NB, Popov DV Quantitative analysis of flavonoids in herbal remedy by high-performance liquid chromatography // Siberian medical journal. - 2013. - No. 1. - P. 114-115.

4. Evdokimova O.V., Samylina I.A., Nesterova O.V. Determination of the content of the amount phospholipids and carotenoids in fruits of some hawthorn species // Pharmacy. - 1992. - No. 6. - P. 70 - 72.

5. Muravyov I.A., Shatilo V.V., Semenchenko V.F. Spectrophotometric method quantitative determination of ursolic acid // Chemistry of natural compounds. - 1972. - No. 6. - P. 738–740.

6. Govindarajan R., Rastogi S., Vijayakumar M. Studies on the antioxidant activities of Desmodium gagenticum // Biol. Pharm. Bull. - 2003. - V. 26. - P. 1424-1427.

7. Olennikov D.N., Zilfikarov I.N., Toropova A.A. and others. Chemical composition of callisia juice fragrant (Callisia fragrans Wood.) and its antioxidant activity (in vitro) // Chemistry of vegetable raw materials. - 2008. - No. 4. - P. 95–100.

8. Chen A.-S., Taguchi T., Sakai K. et al. Antioxidant activities of chitobiose and chititriose // Biol. Pharm. Bull. - 2003. - 2003. –V. 26. - P. 1326-1330.

9. Klebanov G.I., Babenkova I.V., Teselkina Yu.O. and others. Assessment of antioxidant activity blood plasma with the use of yolk lipoproteins // Laboratornoe delo. - 1988. - No. 5. - S. 59–62.

10. Korolyuk M.A., Ivanova L.I., Mayorova I.G. et al. Method for determining the activity of catalase // Lab. case. - 1988. - No. 1. - P. 16-19.

11. Anderson RL, Linas SL, Berns AS et al. Nonoleguric acute renal failure / // N. Engl. J. Med. - 1977. - V. 236. - P. 1134.

12. Temirbulatov R.A., Seleznev E.I .. Method of increasing the intensity of free radical oxidation of lipid-containing blood components and its diagnostic value // Lab. case. - 1981. - No. 4. - P. 209–211.

Author's address
Lubsandorzhieva P.B.
IOEB SB RAS, laboratory of biomedical research

bpunsic@mail.ru

Study of the phenolic composition and antioxidant activity of the adaptogenic agent "Adaptofit" in vitro and in vivo / P.B. Lubsandorzhieva, M.V. Baldandorzhieva, L.N. Shantanova, L.V. Osadchuk // Traditional medicine. - 2014. - No. 3 (38). - S.36-40.