UDC 615.322+581.6:581.192

# APPLICATION OF BITTER WORMWOOD IN TREATMENT OF ONCOLOGICAL DISEASES

**A.M. REDKA, M.M. BARANOVSKY**

National Aviation University, Kyiv

Purpose of the work was to describe antitumor activity of biologically active substances of bitter wormwood, the possibility of drug creating on its basis and bitter wormwood application in the complex treatment of oncological diseases. The influence of extractive substances of bitter wormwood on the M-NFS-60 and WISH cell lines was considered as well. The cell culture method based on cultivating of M-NFS-60 and WISH cell lines with an aqueous test extract of bitter wormwood in a 5 % $CO_2$ atmosphere and live cells quantifying by fluorescence spectroscopy was considered in the article. It was found, that with an increase in the concentration of aqueous extract of bitter wormwood, cells M-NFS-60 and WISH die or lose their reproductive capacity. At 0,1 % concentration of aqueous extract the biologically active substances of bitter wormwood cause death of M-NFS-60 cells and loss of their reproductive capacity by 52,2 % (for number 1) and 52 % (for number 2); at 0,05% concentration by 44,7 % and by 47,4 %, respectively. With a decrease in the concentration of aqueous extract of bitter wormwood to 0,0016 %, cell survival is increasing and gradually reaching 100 %. At 0,1% concentration of aqueous extract of bitter wormwood it biologically active substances cause death of WISH cells and loss of their reproductive capacity by 78,6 % (for number 1) and 80,5 % (for number 2); at 0,05 % concentration – by 53,7 % and 60,3 % respectively. With a decrease in the concentration of aqueous extract of wormwood to 0,0016 %, cell survival is increasing and gradually reaching 100 %.

***Key words*:** extraction, cell line, biologically active substances, cultivation, inhibition**.**

**Introduction.** The main task of the pharmacognosy nowadays is to find appropriate plant sources of biologically active substances among the representatives of Ukrainian flora and to create effective medicines on their basis. Valuable sources of biologically active substances are species of the genus *Artemisia* L. of the *Asteraceae* family.

One of the most dangerous disease that has been developed last century is cancer. Recent statistics show that cancer is leading cause of death, accounting for more than 15 % of all deaths.

In the treatment of malignant neoplasms, chemotherapy is prescribed, which has a detrimental effect not only on dangerous cancer cells, but also on healthy ones. Many researchers and scientists have shown in their experiments that bitter wormwood destroys one healthy cell for every 12,000 infected, while in result of chemotherapy 1 normal cells per 10–15 cancer cells die.

Genus *Artemisia* has about 500 species globally and more than 30 species are representedin the flora of Ukraine. Bitter wormwood *Artemisia absinthium* L. is the only official species included in the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

It was scientifically suggested, that sesquiterpene alcohols and lactones, bitter glycosides, flavonoids, alkaloids, vitamins and organic acids represent biologically active substances of wormwoods.

Due to the wide range of biologically active substances, the genus *Artemisia* is the object of a comprehensive study to develop drugs not only with antimicrobial, cardiotonic, anti-inflammatory, choleretic, immunostimulating, but also with antitumor activity.

*Artemisia absinthium* is a herbaceous perennial plant with fibrous roots. The stems are straight, growing to 0.8–1.2 meters (2 ft 7 in–3 ft 11 in) (rarely 1.5 m, but, sometimes even larger) tall, grooved, branched, and silvery-green. The leaves are

spirally arranged, greenish-grey above and white below, covered with silky silvery-white trichomes, and bearing minute oil-producing glands. The basal leaves are up to 25 cm long, bipinnate to tripinnate with long petioles, with the cauline leaves (those on the stem) smaller, 5–10 cm long, less divided, and with short petioles; the uppermost leaves can be both simple and sessile (without a petiole). Its flowers are pale yellow, tubular, and clustered in spherical bent-down heads (capitula), which are in turn clustered in leafy and branched panicles. Flowering is from early summer to early autumn; pollination is anemophilous. The fruit is a small achene; seed dispersal is by gravity [1].

**Materials and methods of research.** As a sensitive culture, a cell line M-NFS-60 (ATCC, № CRL-1838) and WISH (ATCC, CCL-250) was used (Table 1).

*Table 1*

**Cell lines M-NFS-60 and WISH features [2-3]**

| | **M-NFS-60** | **WISH** |
|---|---|---|
| **Organism** | *Musmusculus*,mouse | *Homo sapiens*, human |
| **Tissue** | Blood | HeLa contaminant |
| **Morphology** | Lymphoblast | Epithelial |
| **Growth properties** | Suspension | Adherent |
| **Description** | A murine myeloblastic cell line established from leukemic cells obtained after infection of (NFS X DBA/2) F1 adult mice with Cas Br-M murine leukemia virus. NFS-60 cells are dependent on IL3 for growth and maintenance of viability in vitro. | Human papillomavirus-related endocervical adenocarcinoma |

Figure 1 represents cell lines M-NFS-60 (1) and WISH (2) through microscope.

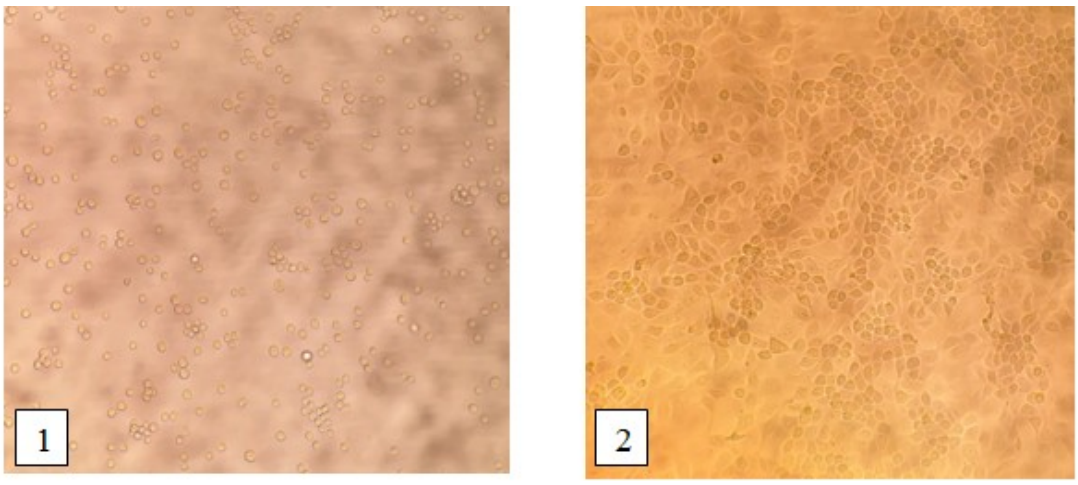


**Fig. 1. Cell lines M-NFS-60 (1) and WISH (2) through microscope**

RPMI-1640 is a form of medium used in cell culture and tissue culture used for growing a variety of mammalian cell lines [4].

For WISH cell cultivation nutrient medium RPMI-1640 (Gibco, Cat. № 21875) was used, to which 1 % antibiotic-antimycotic solution (10,000 IU/ml penicillin, 10 mg/ml streptomycin, 25 µg/ml amphotericin B) (Gibco, No. 15240) and 8–10 % of embryonic bovine serum (Gibco, No. 10500) was added.

For M-NFS-60 cell cultivation nutrient medium RPMI-1640 (Gibco, Cat. № 21875) was used, to which 1 % antibiotic-antimycotic solution (10,000 IU/ml penicillin, 10 mg/ml streptomycin, 25 µg/ml amphotericin B) (Gibco, No. 15240) (Gibco, № 15240) and 1 % of embryonic bovine serum (Gibco, № 10500) was added.

Aqueous extract of bitter wormwood was prepared using dry raw materials produced by two separate manufactures: №1 – "Liktravy", №2 – "Viola". Aqueous extract of bitter wormwood (1 %) was diluted five times. For this purpose, 200 µl of the drug was diluted to 1000 µl volume by nutrient medium and carefully mixed.

Results were recorded by fluorescence spectrophotometry using a microplate reader (Cytation3BioTek) in regime Ex 560 nm, Em 590 nm.

**Results and their discussions.** It has been estimated the difference between average value of negative control of cell state and average Blank value, accounting for 2126 rfu. The percentage of living cells in proportion to aqueous extract of bitter wormwood concentration was determined (Table 2).

*Table 2*

**The percentage of living cells after culturing with aqueous extract of bitter wormwood**

| **Concentration** | | **Emission value, rfu** | | | | | **% of living cell in respect to K-** | **Standard deviation** | **Coefficient of variation (CV), %** |
|---|---|---|---|---|---|---|---|---|---|
| **%** | **Dilution** | **1** | **2** | **3** | **Average value** | **Average value - Blank** | | | |
| **Test solution №1** | | | | | | | | | |
| **0,1** | 1/10 | 1346 | 1352 | 1360 | 1353 | 1016 | **47,8** | 7,0 | **0,5** |
| **0,05** | 1/20 | 1502 | 1494 | 1539 | 1512 | 1175 | **55,3** | 24,0 | **1,6** |
| **0,025** | 1/40 | 1650 | 1633 | 1711 | 1665 | 1328 | **62,5** | 41,0 | **2,5** |
| **0,0125** | 1/80 | 1921 | 1865 | 1865 | 1884 | 1547 | **72,8** | 32,3 | **1,7** |
| **0,00625** | 1/160 | 2152 | 2142 | 2184 | 2159 | 1823 | **85,7** | 21,9 | **1,0** |
| **0,003125** | 1/320 | 2395 | 2391 | 2465 | 2417 | 2080 | **97,8** | 41,6 | **1,7** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **0,0015625** | 1/640 | 2493 | 2390 | 2451 | 2445 | 2108 | **99,1** | 51,8 | **2,1** |
| **Test solution №2** | | | | | | | | | |
| **0,1** | 1/10 | 1357 | 1381 | 1335 | 1358 | 1021 | **48,0** | 23,0 | **1,7** |
| **0,05** | 1/20 | 1423 | 1514 | 1430 | 1456 | 1119 | **52,6** | 50,6 | **3,5** |
| **0,025** | 1/40 | 1534 | 1609 | 1572 | 1572 | 1235 | **58,1** | 37,5 | **2,4** |
| **0,0125** | 1/80 | 1801 | 1842 | 1868 | 1837 | 1500 | **70,6** | 33,8 | **1,8** |
| **0,00625** | 1/160 | 2064 | 2130 | 2065 | 2086 | 1750 | **82,3** | 37,8 | **1,8** |
| **0,003125** | 1/320 | 2368 | 2463 | 2371 | 2401 | 2064 | **97,1** | 54,0 | **2,2** |
| **0,0015625** | 1/640 | 2475 | 2517 | 2578 | 2523 | 2187 | **102,8** | 51,8 | **2,1** |

Using emission values for each concentration level of tested solutions was determined coefficient of variation (CV, %). Coefficient of variation characterizes fluctuations (variability) of the found emission values. CV must not exceed 15 %. The maximum value of the coefficient of variation is 3,5 %.

The difference between average value of negative control of cell state and average Blank value has been estimated, accounting for 7076 rfu. The percentage of living cells in proportion to aqueous extract of bitter wormwood concentration was determined (Table 3–4).

*Table 3*

**The percentage of living cells after culturing with aqueous extract of bitter wormwood №1**

| **Concentration** | | **Emission value, rfu** | | | | | **% of living cell in respect to K-** | **Standard deviation** | **Coefficient of variation (CV), %** |
|---|---|---|---|---|---|---|---|---|---|
| **%** | **Dilution** | **1** | **2** | **3** | **Average value** | **Average value - Blank** | | | |
| **Test solution №1** | | | | | | | | | |
| **0,1** | 1/10 | 2160 | 2120 | - | 2140 | 1514 | **21.4** | 28 | **1,3** |
| **0,05** | 1/20 | 3956 | 3989 | 3756 | 3900 | 3274 | **46,3** | 126 | **3,2** |
| **0,025** | 1/40 | 5896 | 6140 | 5828 | 5955 | 5329 | **75,3** | 164 | **2,8** |
| **0,0125** | 1/80 | 6847 | 6735 | 6693 | 6758 | 6132 | **86,7** | 80 | **1,2** |
| **0,00625** | 1/160 | 7062 | 7133 | 7128 | 7108 | 6482 | **91,6** | 40 | **0,6** |
| **0,003125** | 1/320 | 7366 | 7481 | 7465 | 7437 | 6811 | **96,3** | 62 | **0,8** |
| **0,0015625** | 1/640 | 7721 | 7823 | 7831 | 7792 | 7166 | **101,3** | 61 | **0,8** |

*Table 4*

**The percentage of living cells after culturing with aqueous extract of bitter wormwood №2**

| Concentration | | Emission value, rfu | | | | | % of living cell in respect to K- | Standard deviation | Coefficient of variation (CV), % |
|---|---|---|---|---|---|---|---|---|---|
| % | Dilution | 1 | 2 | 3 | Average value | Average value - Blank | | | |
| **Test solution №2** | | | | | | | | | |
| **0,1** | 1/10 | 2051 | 1897 | 2077 | 2008 | 1382 | **19,5** | 97 | **4,8** |
| **0,05** | 1/20 | 3708 | 3160 | - | 3434 | 2808 | **39,7** | 387 | **11,3** |
| **0,025** | 1/40 | 5486 | 6159 | 5719 | 5788 | 5162 | **73,0** | 342 | **5,9** |
| **0,0125** | 1/80 | 6328 | 6460 | 6538 | 6442 | 5816 | **82,2** | 106 | **1,6** |
| **0,00625** | 1/160 | 7036 | 7241 | 7133 | 7137 | 6511 | **92,0** | 103 | **1,4** |
| **0,003125** | 1/320 | 7499 | 7435 | 7634 | 7523 | 6897 | **97,5** | 102 | **1,4** |
| **0,0015625** | 1/640 | 7715 | 7734 | 7850 | 7766 | 7140 | **100,9** | 73 | **0,9** |

Using emission values for each concentration level of tested solutions was estimated coefficient of variation (CV, %). Coefficient of variation characterizes fluctuations (variability) of the found emission values. CV must not exceed 15 %. The maximum value of the coefficient of variation is 11,3 %.

## CONCLUSIONS

With an increase in the concentration of aqueous extract of *Artemisia*, cells M-NFS-60 die or lose their reproductive capacity. At 0,1 % concentration of aqueous extract the biologically active substances of bitter wormwood cause death of cells and loss of their reproductive capacity by 52,2 % (for number 1) and 52 % (for number 2); at 0,05 % concentration – by 44,7 % and by 47,4 %, respectively. With a decrease in the concentration of aqueous extract of bitter wormwood to 0,0016 %, cell survival is increasing and gradually reaching 100 %.

With an increase in the concentration of aqueous extract of bitter wormwood, WISH cells die or lose their reproductive capacity. At 0,1 % concentration of aqueous extract of bitter wormwood it biologically active substances cause death of cells and loss of their reproductive capacity by 78,6 % (for number 1) and 80,5 % (for number

2); at 0,05 % concentration – by 53,7 % and 60,3 % respectively. With a decrease in the concentration of aqueous extract of wormwood to 0,0016 %, cell survival is increasing and gradually reaching 100 %.

**REFERENCES:**

1. *Artemisia absinthium* Linnaeus, Sp. Pl. 2: 848. 1753. // Flora of North America. – 2006. – Vol. 19, 20, 21 – P. 519

2. American Type Culture Collection: M-NFS-60 cell line.

3. American Type Culture Collection: WISH cell line.

4. Sigma-Aldrich: RPMI-1640 cell culture.

## ЗАСТОСУВАННЯ ПОЛИНУ ГІРКОГО У ЛІКУВАННІ ОНКОЗАХВОРЮВАНЬ

**А.М. РЕДЬКА, М.М. БАРАНОВСЬКИЙ**

Національний авіаційний університет, м. Київ

Метою роботи є опис протипухлинної активності біологічно активних речовин Полину гіркого, можливість створення препарату на його основі та застосування у комплексному лікуванні онкологічних захворювань. Розглянуто вплив екстрактивних речовин Полину гіркого на клітинні лінії M-NFS-60 та WISH. У статті розглянуто метод культури клітин, що заснований на культивуванні клітинних ліній M-NFS-60 та WISH із випробовуваним водним екстрактом Полину гіркого у атмосфері 5 % $CO_2$ та кількісному визначенні живих клітин методом флуоресцентної спектроскопії. Встановлено, що зі збільшенням концентрації водного екстракту полину гіркого клітини M-NFS-60 та WISH гинуть або втрачають здатність до розмноження. При концентрації водного екстракту 0,1 % біологічно активні речовини полину гіркого спричиняють загибель клітин M-NFS-60 та втрату здатності до розмноження на 52,2 % (для №1) та на 52 % (для №2); при концентрації 0,05 % – на 44,7 % та на

47,4 % відповідно. Зі зменшенням концентрації водного екстракту полину гіркого до 0,0016 % виживаність клітин зростає і поступово досягає 100 %. При концентрації водного екстракту 0,1 % біологічно активні речовини полину гіркого спричиняють загибель клітин WISH та втрату здатності до розмноження на 78,6 % (для №1) та на 80,5 % (для №2); при концентрації 0,05 % – на 53,7 % та на 60,3 % відповідно. Зі зменшенням концентрації водного екстракту полину гіркого до 0,0016 % виживаність клітин зростає і поступово досягає 100 %.

**Ключові слова:** екстракція, клітинна лінія, біологічно активні речовини, культивування, інгібування.

## ПРИМЕНЕНИЕ ПОЛЫНИ ГОРЬКОЙ В ЛЕЧЕНИИ ОНКОЗАБОЛЕВАНИЙ

**А.М. РЕДЬКА, М.М. БАРАНОВСЬКИЙ**

Национальный авиационный университет, г. Киев

Целью работы является описание противоопухолевой активности биологически активных веществ полыни горькой, возможность создания препарата на его основе и применение в комплексном лечении онкологических заболеваний. Рассмотрено влияние экстрактивных веществ Полыни горькой на клеточные линии M-NFS-60 и WISH. В статье рассмотрен метод культуры клеток, основанный на культивировании клеточных линий M-NFS-60 и WISH с испытуемым водным экстрактом Полыни горькой в атмосфере 5 % $CO_2$ и количественном определении живых клеток методом флуоресцентной спектроскопии. Установлено, что с увеличением концентрации водного экстракта Полыни горькой клетки M-NFS-60 и WISH погибают или теряют способность к размножению. При концентрации водного экстракта 0,1 % биологически активные вещества полыни горькой вызывают гибель клеток M-NFS-60 и потерю способности к размножению на 52,2 % (для №1) и на 52 %

(для №2); при концентрации 0,05 % – на 44,7 % и на 47,4 % соответственно. С уменьшением концентрации водного экстракта полыни горькой к 0,0016 % выживаемость клеток растет и постепенно достигает 100 %. При концентрации водного экстракта 0,1 % биологически активные вещества Полыни горькой вызывают гибель клеток WISH и потерю способности к размножению на 78,6 % (для №1) и на 80,5 % (для №2); при концентрации 0,05 % – на 53,7 % и на 60,3 % соответственно. С уменьшением концентрации водного экстракта полыни горькой к 0,0016 % выживаемость клеток растет и постепенно достигает 100 %.

**Ключевые слова:** экстракция, клеточная линия, биологически активные вещества, культивирование, ингибирование.