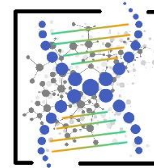
Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com



EVALUATION OF CYTOTOXIC AND CYTOSTATIC ACTIVITY OF *CHLOROPHYTUM COMOSUM* METHANOL EXTRACT

Murad Elshanovich Turabov[1], Victoria Viktorovna Kolesnikova[1], Aziza Rasulovna Bazaeva[2], Dina Bislanovna Dzukheraeva[3], Vladimir Vladimirovich Ternovoy[1], Aleksandr Dmitrievich Kompaneec[1], Viktoria Aleksandrovna Zhiteneva[4], Vera Arturovna Medvedeva[4*]

1. ***Department of Therapy, Faculty of Medicine of Stavropol State Medical University, Stavropol, Russia***
2. ***Department of Therapy, Faculty of Pediatrics of Stavropol State Medical University, Stavropol, Russia***
3. ***Department of Therapy, Faculty of Medicine, North Ossetian State Medical Academy, Vladikavkaz, Republic of North Ossetia-Alania, Russia***
4. ***Department of Therapy, Faculty of Dentistry, Private Educational organization of Higher Professional Education "Reaviz Medical University", Moscow, Russia.***

ARTICLE INFO

Received:
07 March 2024
Received in revised form:
02 July 2024
Accepted:
09 July 2024
Available online:
28 August 2024

Keywords: *Chlorophytum comosum*, Flavonoids, SPEV, Rat liver cancer, Ecotoxicology, Traditional medicine

ABSTRACT

Chlorophytum comosum leaf extract is well-known to contain flavonoids. The extract has cytotoxic and cytostatic activity in normal animal cells. At concentrations of 15 mg/mL and above, the extract leads to 100% death of SPEV cells. The number of dead cells of the SPEV line generally increases with increasing extract concentration. The LC50 of *Chlorophytum comosum* extract was determined by probit analysis (LC50 = 7.24 mg/mL). With an increase in the concentration of the extract, a noticeable decrease in the proliferative activity of cells was observed. After 48 hours, the inhibition of proliferative activity of SPEV cells is more pronounced than after 24 hours. The percentage of SPEV cells that died under the action of *Chlorophytum comosum* extract becomes smaller with increasing exposure time. The results of the analysis of the tumor mass at the end of the experiment fully correspond to the dynamics of its growth. The data obtained by us indicate the absence of the effect of *Chlorophytum comosum* extract against the transferable liver cancer of rats in an in vivo experiment.

*This is an **open-access** article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, and build upon the work non commercially.*

To Cite This Article: Turabov ME, Kolesnikova VV, Bazaeva AR, Dzukheraeva DB, Ternovoy VV, Kompaneec AD, et al. Evaluation of Cytotoxic and Cytostatic Activity of *Chlorophytum comosum* Methanol Extract. Pharmacophore. 2024;15(4):33-8. https://doi.org/10.51847/D5CauwttEJ

Introduction

Chlorophytum comosum belongs to poisonous plants due to the content of aristolochiic acid in the leaves [1]. However, in traditional medicine, this plant is still used to treat arthritis, facilitate childbirth, as part of weight loss supplements, treat rheumatism and stabilize the menstrual cycle, and as an antitumor agent [2-4]. However, there is a lack of scientific data on antitumor activity and its mechanisms [5, 6].

Recent studies show that *Chlorophytum comosum* extracts containing aristolochiic acid have pronounced carcinogenic and mutagenic effects [7, 8]. Therefore, the International Agency for Research on Cancer has classified *Chlorophytum comosum* as the first class of oncogenes that cause cancer in humans and animals [9]. The relationship between *Chlorophytum comosum* extracts containing aristolochiic acid and cancer of the upper urinary tract and kidneys was noted. Thus, about 1,500 genes with mutations, including mutations of the P53 gene, were found in malignant tumors in patients taking such extracts [10-12]. However, Rzhepakovsky *et al*. [13] developed a method for obtaining *Chlorophytum comosum* extract that significantly reduces its toxicity and, at the same time, provides a greater yield of flavonoids. Notably, other studies also revealed the presence of several positive pharmacological values in poisonous plant extracts containing flavonoids, including antitumor,

Corresponding Author: Vera Arturovna Medvedeva; Department of Therapy, Faculty of Dentistry, Private Educational organization of Higher Professional Education "Reaviz Medical University", Moscow, Russia. E-mail: bucky99@ya.ru.

anticachexic, anti-tuberculosis, anti-inflammatory, antipyretic, antimicrobial, and genoprotective activity [14-17]. According to Li *et al*. [18] leaves of *Chlorophytum comosum* contain such flavonoids as quercetin glycoside, quercetin (in hydrolysate), the flowers contain quercetin glycoside, and two flavonol glycosides. The data obtained earlier indicate the absence of toxicity and weak cytogenetic activity of the *Chlorophytum comosum* methanol extract [19].
Thus, this work aimed to study the activity of the flavonoid-containing extract of *Chlorophytum comosum* against the SPEV cells *in vitro* and to establish the presence or absence of its antitumor activity against transferable rat liver cancer *in vivo.*

Materials and Methods

An aqueous solution of dry methanol extract of *Chlorophytum comosum* leaves was used in this study. *Chlorophytum comosum* was grown in the laboratory greenhouse complex of St. Petersburg State Agrarian University (St. Petersburg, Russia). The extract was obtained by the method of Rzhepakovsky *et al*. [13].
The culture of pig embryo kidney SPEV cells was taken from the cryobank of the cell culture collection of the laboratory of the North Ossetian State Medical Academy (Vladikavkaz, Russia). The cell culture was grown in RPMI 4 medium (10% embryonic serum, gentamicin, ampicillin, amphotericin) in 6-well plates according to the method of Elati *et al*. [20]. The cells in the medium without the extract served as a control. An extract solution diluted in a nutrient medium with varied concentrations of 30, 15, 7.5, 3.75, 1.85, and 0.9375 mg/mL was introduced into six wells. Then, SPEV cells were cultured in a CO_2 incubator at 37 °C for 24 hours. Propidium iodide was used as a dye, which penetrates cells by destroying the membrane and binds to nuclear DNA [21]. To visualize the SPEV cells, a combination of several microscopic modes of light scattering and fluorescence detection on a Leica DM 2500 microscope, a Leica DFC 420C digital video camera with Leica Application Suite V 3.1 software was used.
Photographing was carried out in different modes: in phase contrast (allowing analysis of the surface of SPEV cells), at fluorescent illumination (allowing to count the number of dead SPEV cells by glowing red nuclei colored with propidium iodide), in normal light mode (allowing to visualize dead SPEV cells with living ones colored green with acridine orange) [22]. The following indicators were used in the experiment: the total number of SPEV cells in the field of view, the number of dead SPEV cells, and the number of living SPEV cells. Probit analysis was used to calculate the LC50 of the extract about SPEV cells [23].
An *in vivo* experiment was carried out in accordance with the Guide for the Care and Use of Laboratory Animals [24]. For the experiment, 12 male white laboratory rats weighing 150 ± 50 g were implanted subcutaneously in the scapula area with 0.5 ml of 25% tumor suspension in Hanks solution of the strain of alveolar liver cancer (PC-1), obtained from the bank of tumor strains of the North Ossetian State Medical Academy (Vladikavkaz, Russia). Animals with transferable cancer were randomly divided into two groups of 6 rats. In the experimental group, the solution was administered intramuscularly to rats at a dosage of 100 mg/kg, once a day for 16 days from the moment of tumor transplantation. After the withdrawal of the extract, animal observations continued for another week. The dynamics of tumor growth was assessed by changing its volume according to the Eq. 1 [25]:

$$V = A \times B \times C \tag{1}$$

where A is the width, B is the thickness, and C is the height of the tumor.
Measurements were carried out with an electronic vernier caliper every two days from the beginning of the experiment. On the 23rd day, the rats were removed from the experiment, and samples of organ tissue, tumors, and blood were taken for additional studies.
Statistical data processing was carried out in the STATISTICA 12.0 software using methods of biomedical statistics. The significance of differences in the parametric distribution was determined using the Mann-Whitney criterion for independent samples at $P < 0.005$ [26].

Results and Discussion

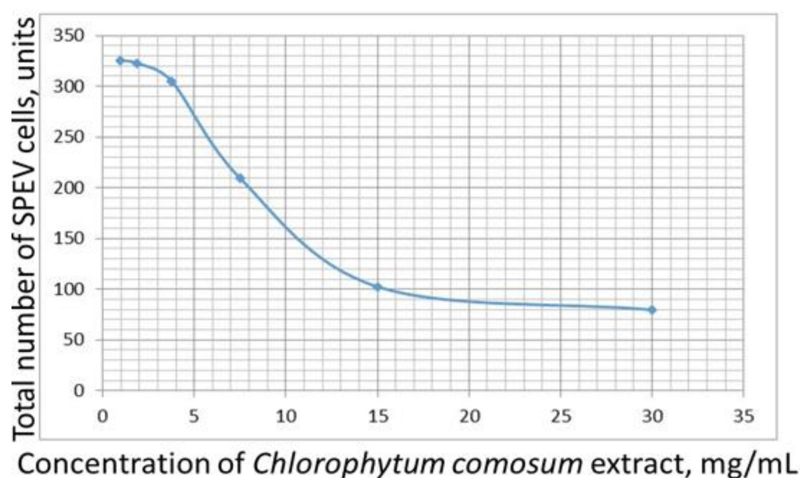
Investigation of the activity of a flavonoid-containing extract of *Chlorophytum comosum* against SPEV cells showed an interesting result. The control included living polygonal cells with 2-3 nucleoli. There was a visible uniform increase in the number of cells and the formation of a monolayer within 24 hours. The complete monolayer was formed in 48 hours. When exposed to the *Chlorophytum comosum* extract, compared with the control, there was a significant increase in the number of SPEV cells not attached to the substrate. Notably, the shape of SPEV cells under extract treatment changed from polygonal to rounded. Statistically significant differences in cell culture under the action of the extract were revealed at all concentrations compared with the control **(Table 1)**.

Table 1. Comparison of the number of SPEV cells in experimental groups

Experimental groups	Total number of SPEV cells, un.	Number of dead SPEV cells, un.	Number of living SPEV cells, un.
24 hours			
Control group	481.3±130.90	2.33±1.500	479.0±130.09
Experimental group (30 mg/mL)	79.75±72.06 *	79.75±72.06 *	0.0±0.00
Experimental group (15 mg/mL)	102.5±50.68	102.5±50.68 *	0.0±0.00
Experimental group (7.5 mg/mL)	209.5±74.77 *	114±48.9 *	95.5±31.45 *
Experimental group (3.75 mg/mL)	305.25±54.020	31.0±17.16	274.25±38.48 *
Experimental group (1.875 mg/mL)	322.75±38.600	38.25±26.110	31.0±27.30 *
Experimental group (0.9375 mg/mL)	325.75±77.070	41.25±29.680 *	284.5±53.03 *
48 hours			
Control group	461±110.9	9.0±9.11	452.0±109.66
Experimental group (1.875 mg/mL)	49±6.0 *	6.66±7.200 *	42.33±4.040 *

* $P \leq 0.005$

According to **Table 1**, at ≥15 mg/mL *Chlorophytum comosum* extract, 100% SPEV cell death occurred after 24 hours. Under the action of *Chlorophytum comosum* extract, the total number of SPEV cells decreased at all concentrations **(Figure 1a)**, which indicates the suppression of the proliferative activity of non-tumor SPEV cells [27]. With an increase in the concentration of *Chlorophytum comosum* extract, a noticeable decrease in the proliferative activity of cells was observed.



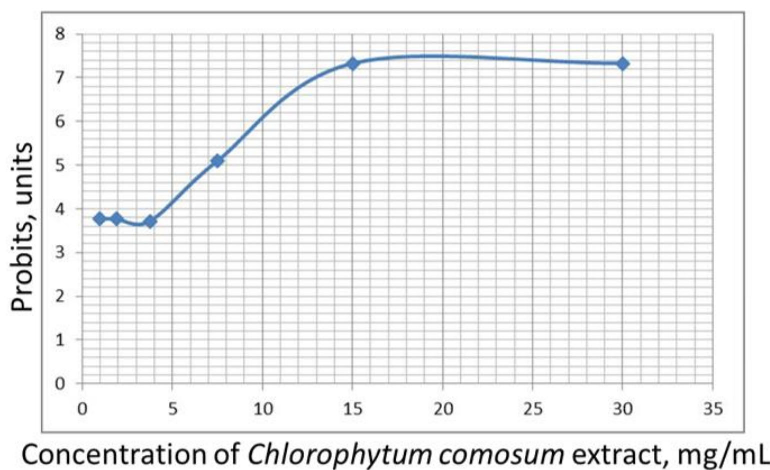
a)

Exposition time, h	Control group	Experiment al group
24	481.3±130.9	322.75±38.6
48	461±110.9	49±6.0

b)

Figure 1. Assessment of the total number of SPEV cells: dependence of the total number of SPEV cells on concentration of *Chlorophytum comosum* extract (a) and effect of 1.875 mg/mL extract of *Chlorophytum comosum* on SPEV cells after 24 and 48 hours of exposition (b).

After 48 hours, the inhibition of the proliferative activity of SPEV cells was more pronounced than after 24 hours **(Figure 1b)**. Interestingly, the number of dead SPEV cells generally increases with an increase in the concentration of *Chlorophytum comosum* extract **(Figure 2a)**. The LC50 of *Chlorophytum comosum* extract was 7.24 mg/mL. It is worth noting, that the percentage of SPEV cells that died under the action of *Chlorophytum comosum* extract becomes smaller with increasing exposure time **(Figure 2b)**.



a)

Exposition time, h	Dead SPEV cells,%
24	10.92
48	10.79

b)

Figure 2. Assessment of SPEV cells viability: dependence of the number of dead SPEV cells on concentration of *Chlorophytum comosum* extract (a) and influence of 1.875 mg/mL extract of *Chlorophytum comosum* on number of dead SPEV cells after 24 and 48 hours of exposition (b).

A study of the biological activity of the flavonoid-containing extract of *Chlorophytum comosum* in relation to transferable rat liver cancer *in vivo* revealed interesting results **(Figure 3)**. In the control, a noticeable tumor growth was observed on day 11, which gradually increased until the end of the experiment. In the group of rats with an overgrown tumor under the action of the extract, tumor growth did not differ from the control until the 16th day of observation, and starting from the 17th day it became higher than in the control.

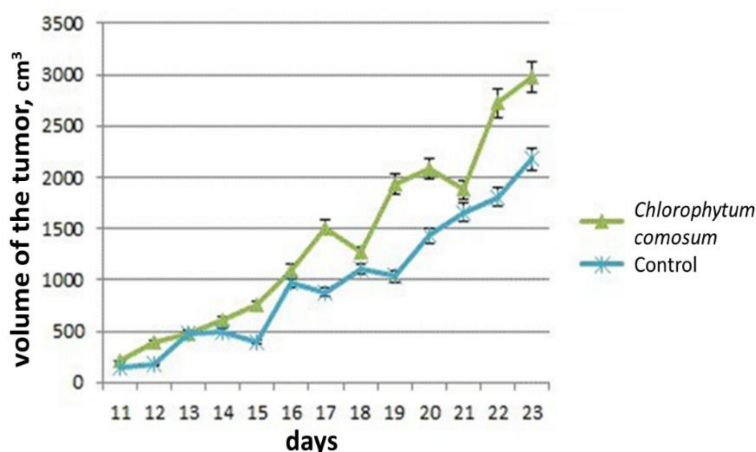


Figure 3. Dynamics of growth of the PC-1 transplanted tumor of rats under the action of *Chlorophytum comosum* extract.

The results of the analysis of the tumor mass at the end of the experiment fully correspond to the dynamics of its growth. The mass of the tumor practically did not differ from the control, which means that the extract we studied in the selected dosage does not have antitumor activity [28]. The data obtained by us indicate the absence of the effect of *Chlorophytum comosum* extract on the transplanted tumor PC-1.

Conclusion

Thus, the flavonoid-containing extract of *Chlorophytum comosum* does not have antitumor activity against PC-1 transferable liver cancer of rats and, considering the revealed cytotoxicity against non-tumor SPEV cells, it is likely to prove unpromising for further investigation of its antitumor activity against other types of tumors.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: The work with laboratory animals was carried out in accordance with the research protocol, which does not contradict the Geneva Convention of 1985 on "International Principles of Biomedical Research using Animals". The topic and descriptions of the experiments were approved by the Ethical Commission of the North Ossetian State Medical Academy (Vladikavkaz, Russia) (Protocol No. 13 of November 3, 2023).

References

1. Gawrońska H, Bakera B. Phytoremediation of particulate matter from indoor air by *Chlorophytum comosum* L. plants. Air Qual Atmos Health. 2015;8(3):265-72. doi:10.1007/s11869-014-0285-4
2. Peng YJ, Cui XY, Tan MC, Hu L, Ruan HY, Shao YY, et al. Characterization of the complete chloroplast genome of *Chlorophytum comosum* (Liliaceae). Mitochondrial DNA B Resour. 2020;5(1):1046-7. doi:10.1080/23802359.2020.1721362
3. Azami AI, O'Neill K, Pirro S, Ghazal H. The complete genome sequence of *chlorophytum comosum* (Asparagaceae, Asparagales), the spider plant. Biodivers Genomes. 2022;2022:10.56179/001c.55588. doi:10.56179/001c.55588
4. Rzhepakovsky I, Anusha Siddiqui S, Avanesyan S, Benlidayi M, Dhingra K, Dolgalev A, et al. Anti-arthritic effect of chicken embryo tissue hydrolyzate against adjuvant arthritis in rats (X-ray microtomographic and histopathological analysis). Food Sci Nutr. 2021;9(10):5648-69. doi:10.1002/fsn3.2529
5. Adhami S, Farooqi H, Abdin MZ, Prasad R, Malik AA. Chemical profiling of chlorophytum comosum (Thunb.) Jaques by GC-MS/LC-ESIMS and its antiproliferative effects on human carcinoma cell lines. Anticancer Agents Med Chem. 2021;21(13):1697-707. doi:10.2174/1871520620666201123085300
6. Padayachee B, Odun-Ayo F, Reddy L. Apoptotic effect of bulbine natalensis and chlorophytum comosum in myelogenous leukemia K562 cell line. Braz J Biol. 2021;84:e251336. doi:10.1590/1519-6984.251336
7. Setsungnern A, Treesubsuntorn C, Thiravetyan P. Chlorophytum comosum-bacteria interactions for airborne benzene remediation: Effect of native endophytic Enterobacter sp. EN2 inoculation and blue-red LED light. Plant Physiol Biochem. 2018;130:181-91. doi:10.1016/j.plaphy.2018.06.042
8. Munyao JN, Dong X, Yang JX, Mbandi EM, Wanga VO, Oulo MA, et al. Complete chloroplast genomes of *chlorophytum comosum* and *chlorophytum gallabatense*: Genome structures, comparative and phylogenetic analysis. Plants (Basel). 2020;9(3):296. doi:10.3390/plants9030296
9. Loomis D, Guha N, Hall AL, Straif K. Identifying occupational carcinogens: An update from the IARC monographs. Occup Environ Med. 2018;75(8):593-603. doi:10.1136/oemed-2017-104944
10. Vakele Y, Odun-Ayo F, Reddy L. In vitro antioxidant and cytotoxicity activities of selected indigenous South African medicinal plants. Afr Health Sci. 2022;22(1):395-403. doi:10.4314/ahs.v22i1.48
11. Qiao S, Song L, Li S, Liu L, Cai H, Si L, et al. Overexpression of CcFALDH from spider plant (*Chlorophytum comosum*) enhances the formaldehyde removing capacity of transgenic gloxinia (Sinningia speciosa)1. Environ Res. 2023;223:115466. doi:10.1016/j.envres.2023.115466
12. Skłodowska M, Świercz-Pietrasiak U, Krasoń M, Chuderska A, Nawrocka J. New insight into short time exogenous formaldehyde application mediated changes in *chlorophytum comosum* L. (Spider Plant) cellular metabolism. Cells. 2023;12(2):232. doi:10.3390/cells12020232
13. Rzhepakovsky IV, Areshidze DA, Avanesyan SS, Grimm WD, Filatova NV, Kalinin AV, et al. Phytochemical Characterization, antioxidant activity, and cytotoxicity of methanolic leaf extract of *chlorophytum comosum* (Green Type) (Thunb.) Jacq. Molecules. 2022;27(3):762. doi:10.3390/molecules27030762
14. Gupta T, Kataria R, Sardana S. A comprehensive review on current perspectives of flavonoids as antimicrobial agent. Curr Top Med Chem. 2022;22(6):425-34. doi:10.2174/1568026622666220117104709
15. González A, Casado J, Lanas Á. Fighting the antibiotic crisis: Flavonoids as promising antibacterial drugs against *helicobacter pylori* infection. Front Cell Infect Microbiol. 2021;11:709749. doi:10.3389/fcimb.2021.709749
16. Wang L, Chen M, Lam PY, Dini-Andreote F, Dai L, Wei Z. Multifaceted roles of flavonoids mediating plant-microbe interactions. Microbiome. 2022;10(1):233. doi:10.1186/s40168-022-01420-x
17. Ivyna de Araújo Rêgo R, Guedes Silvestre GF, Ferreira de Melo D, Albino SL, Pimentel MM, Silva Costa Cruz SB, et al. Flavonoids-rich plant extracts against *helicobacter pylori* infection as prevention to gastric cancer. Front Pharmacol. 2022;13:951125. doi:10.3389/fphar.2022.951125
18. Li J, Zhong J, Liu Q, Yang H, Wang Z, Li Y, et al. Indoor formaldehyde removal by three species of Chlorophytum comosum under dynamic fumigation system: Part 2-plant recovery. Environ Sci Pollut Res Int. 2021;28(7):8453-65. doi:10.1007/s11356-020-11167-3

19. Setsungnern A, Treesubsuntorn C, Thiravetyan P. Exogenous 24-epibrassinolide enhanced benzene detoxification in Chlorophytum comosum via overexpression and conjugation by glutathione. Sci Total Environ. 2019;662:805-15. doi:10.1016/j.scitotenv.2019.01.258
20. Elati K, Zweygarth E, Mhadhbi M, Darghouth MA, Nijhof AM. Cultivation, cryopreservation, and resuscitation of *Theileria annulata* transformed cells in serum-free media. Front Vet Sci. 2022;9:1055022. doi:10.3389/fvets.2022.1055022
21. Pereira LM, Portapilla GB, Brancini GTP, Possato B, Bronzon da Costa CM, Abreu-Filho PG, et al. The potential of phenothiazinium dyes as cytotoxicity markers in cisplatin-treated cells. Sci Rep. 2023;13(1):10203. doi:10.1038/s41598-023-36721-0
22. Park JH, Sung Y, Jo S, Lee SH, Ryu JH, Sun IC, et al. Applications of Cu2+-loaded silica nanoparticles to photothermal therapy and tumor-specific fluorescence imaging. J Funct Biomater. 2024;15(4):81. doi:10.3390/jfb15040081
23. Gomez DC, Anacta N. A new method to test molluscicides against the philippine schistosomiasis snail vectors. J Parasitol Res. 2020;2020:3827125. doi:10.1155/2020/3827125
24. National Research Council (US). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US); 2011. PMID: 21595115.
25. Ira JI, Islam MS, Misra JC, Kamrujjaman M. Mathematical modelling of the dynamics of tumor growth and its optimal control. Int J Ground Sediment Water. 2020;11:659-79. doi:10.5281/ZENODO.4275629
26. Tai KY, Dhaliwal J, Balasubramaniam V. Leveraging Mann-Whitney U test on large-scale genetic variation data for analyzing malaria genetic markers. Malar J. 2022;21(1):79. doi:10.1186/s12936-022-04104-x
27. Afify SM, Sanchez Calle A, Hassan G, Kumon K, Nawara HM, Zahra MH, et al. A novel model of liver cancer stem cells developed from induced pluripotent stem cells. Br J Cancer. 2020;122(9):1378-90. doi:10.1038/s41416-020-0792-z
28. Shitara K, Falcone A, Fakih MG, George B, Sundar R, Ranjan S, et al. Efficacy and safety of trifluridine/tipiracil-containing combinations in colorectal cancer and other advanced solid tumors: A systematic review. Oncologist. 2024;29(5):e601-15. doi:10.1093/oncolo/oyae007