

ФАРМАЦЕВТИЧНІ НАУКИ

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EVALUATION OF IN VITRO ANTICANCER ACTIVITY OF ACORUS CALAMUS L. AND CALENDULA OFFICINALIS L.

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Acorus calamus has been used for centuries as a folk medicine to treat infectious and noninfectious diseases. Phytopharmacological studies of different *Calendula* extracts have shown antiinflammatory, antibacterial, antiviral properties of therapeutic interest. However, little is known about their anticancer effect. In this study, was evaluated the in vitro anticancer activity of water extracts of the plants *Acorus calamus* and *Calendula officinalis* (Asteraceae). The cytotoxic activity of extracts was performed by MTT assay against murine myeloblastic cell line (NFS-60).

Keywords: *Acorus calamus*, *Calendula officinalis*, extract, anticancer, NFS-60, MTT assay.

Formulation of problem. It has been opined based on global statistics that over 20 million new cancer cases and more than 17 million cancer deaths are probable to occur by the year 2050. Anticancer therapies have largely involved the use of chemical and drugs as a part of chemotherapy against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacy due to the development of various side effects. Therefore, it seems necessary to find new and more effective drugs for cancer therapy. The rate of death by cancers and the destructive side effects of chemical anti-cancer drugs are the major reasons which motivated the researchers to find new, more effective anti-cancer drugs with fewer side effects [1].

One of the most important sources of new drugs are herbal medicine and in this regard, attention on plant components which have anti-cancer properties has been increased. Herbal medicines, also known as herbalism or botanical medicine has been used as a key role in curing the diseases and it is one of the most reliable alternative candidates for anti-cancer drugs. About 80% of the world population are estimated to use traditional medicine, which is mainly based on plant components, for their primary health care. Medicinal plants possess secondary metabolites which are the main sources of medicinal drugs having curative nature and efficiency in human health care. It is believed that phytochemicals which are obtained from the medicinal plants have many benefits for human health and in recent years they have attracted attention because of their efficacy and cost effectiveness.

Analysis of recent research and publications. Plants have a long history of use in the treatment of cancer. Active principles of *Angelica Gigas*, *Catharanthus roseus*, *Podophyllum peltatum*, *Podophyllum emodii*, *Taxus brevifolia*, *Ocrosia elliptica*, and *Campototheca acuminata* have been used in the treatment of advanced stages of various malignancies in the clinical setting [2]. Furthermore, many phytochemicals with different phar-

macological properties have shown responses for the prevention or treatment of different tumors, e.g., flavones, flavanols, isoflavones, catechins, and taxanes [3]. Numerous drugs are used in cancer chemotherapy but most exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non-tumor cells. These side effects limit the use of chemotherapeutic agents despite their high efficacy in treating target malignant cells. Therefore, the search for alternative drugs that are both effective and non-toxic in the treatment of cancers is an important research line. In fact, increased efforts are being made to isolate bioactive products from medicinal plants for their possible utility in cancer treatment [4].

Acorus calamus (*A. calamus*) which is also known as sweet flag (Araceae family) is a beneficial and medicinal plant that has been used in herbal therapies and human health care preparations in the Asia for a long time. *A. calamus* probably belongs to India and now it is found across Europe, Southern Russia, Asia Minor, China, Japan, Burma, Sri Lanka and Northern USA. This plant is a perennial plant and was spread outside its native area in Asia [5].

A. calamus has a very long history of medicinal use in Chinese and Indian herbal traditions. In India, traditional usage of *A. calamus* in Ayurvedic medicine, is for the treatment of cancer, stomach cramps, gastric troubles and also to overcome the side effects of all hallucinogens. *A. calamus* is an important herb in Ayurvedic medicine and is considered as a «Rejuvenator» for the brain and nervous system and as a treatment for digestive diseases. Popular European books on medicinal herbs suggested *calamus* as a «wonder drug». It has been frequently used in folk medicine as a «nervine,» due to its tranquilizing effect of cis-isoasarone. *A. calamus* extract has been used in traditional Chinese prescription due to its beneficial effects on memory and learning performance; also, anti-aging effect in senescence has been reported for this plant [6]. Various extracts of *A. calamus* have anti-diabetes, anti-proliferative, immunosuppressive, hypolipidemic and anti-carcinogenic effects

and the rhizomes and leaves were found to possess anti-carcinogenic activity in human lymphocytes. Ethanolic extract of rhizome of this plant possesses sedative, analgesic, moderately hypotensive and respiratory depressant properties. Many other activities have been reported from this plant including, anti-fungal, anti-bacterial and anti-inflammatory effects [7].

Calendula officinalis L. (Asteraceae) is an annual herb native to the Mediterranean region. In Europe and America it is cultivated for ornamental and medicinal purposes. It is commonly known as the marigold or maravilla, and its flowers have long been employed in folk therapy [8]. More than 35 properties have been attributed to decoctions and tinctures from the flowers, and these preparations have been considered valuable remedies for burns, bruises, cuts, rashes, skin wounds and other.

The main chemical constituents of *Calendula officinalis* include steroids, terpenoids, free and esterified triterpenic alcohols, phenolic acids, flavonoids (quercetin, rutin, narcissin, isorhamnetin, kaempferol), and other compounds [9].

Phytopharmacological studies of different marigold extracts have shown anti-tumoral, anti-inflammatory, wound healing and antioxidant activities. In clinical studies, marigold was highly efficacious in the prevention of acute dermatitis in cancer patients undergoing postoperative irradiation [10].

Its cytotoxic effect on tumor cell lines *in vitro* and its anticancer efficacy *in vivo* was briefly outlined 20 years ago.

Unsolved aspects of the problem. Despite the beneficial role of this plants against many ailments, their anticancer properties are not well understood. Thus, to elucidate the anticancer properties of *A. calamus* and *Calendula officinalis*, we investigated the potential anticancer activity of water extracts on murine myeloblastic cell line (NFS-60).

The purpose of the article. The aim of the present study was to evaluate the *in vitro* cytotoxic anticancer activity of water extracts of the plants *Acorus calamus* and *Calendula Officinalis*.

Statement of basic material. Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors.

Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemias, generally do not form solid tumors.

So, for the checking anticancer activity of *Acorus calamus* and *Calendula Officinalis* we used cancer cell line NFS-60 (suspension). General characteristics of NFS-60 are represented in the Table 1.

The plant materials (sweet flag rhizome and calendula flowers) were grounded into fine particles

with the size 1-3 mm and extracted with water for about 24 hours at 37°C.

Table 1
General characteristics of NFS-60 (suspension) cell line

Organism	Mouse
Tissue	Blood
Morphology	Lymphoblast
Cell type	leukemia, myeloid
Growth properties	Suspension
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 <i>in vitro</i> .

Source: [12]

Cells (murine myeloblastic cell) were cultured in liquid medium (RPMI1640) supplemented 10% Fetal Bovine Serum (FBS), 1% antibiotic antimycotic, 10% IL-3 and maintained under an atmosphere of 5% CO₂ and 95% air at 37°C.

For testing, cells were plated in 96 well plates (3 × 10⁴ cells/ml), were treated with different concentrations of plants extracts including 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 mg/ml. Control cells were incubated in culture medium only. All concentrations of plants extracts were in four replicates on the same cell batch.

Growth of cancer cells quantitated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-terazolium bromide (MTT) to a blue formazan product [11]. At the end of 48 hours incubation, to the medium in each well was added 0.5% MTT solution (5 mg/ml in phosphate-buffered saline), the plates were incubated for 2 hours under 5% CO₂ and 95% air at 37°C. MTT reagent was removed and the formazan crystals produced by viable cells were dissolved in buffer for extraction for 2 hours at 37°C and gently shaken (450 rpm). The absorbance was then determined by Cytation3 reader at 570 nm. The effect of the extracts on the proliferation of NFS-60 was expressed as the% cell inhibition.

The result of investigation shows that extracts of *Acorus calamus* and *Calendula Officinalis* have such cytotoxic activity against NFS-60 (suspension) cell line (Table 2, 3 and Fig 1, 2).

Table 2
Anticancer activity of *Acorus calamus* against NFS-60 (suspension) mouse myeloid cell line

	Dilution	Concentration (mg/ml)	Absorbance (O.D)	Cell inhibition (%)
A	K+	100	0,043	92,32
B	1:2	50	0,046	91,79
C	1:4	25	0,095	83,04
D	1:8	12,50	0,246	56,07
E	1:16	6,25	0,373	33,39
F	1:32	3,12	0,452	19,29
G	1:64	1,56	0,503	10,18
H	K-	Cell control	0,560	0,00

Source: developed by the authors

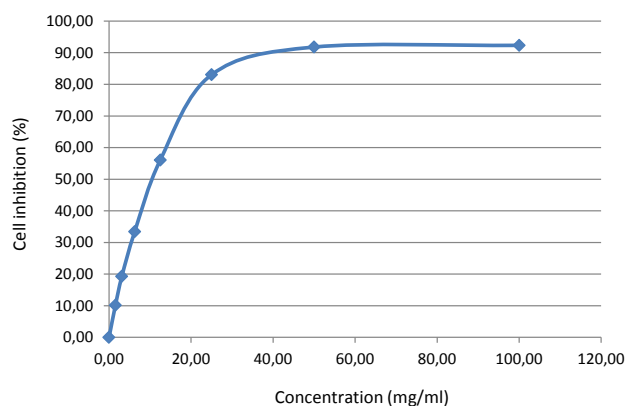


Fig. 1. Anticancer effect of Acorus calamus extract on NFS-60 cell line

Source: developed by the authors

cell inhibition. From the graphs the concentration of water extracts yields the value of IC50 (50% mortality, the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells) as 11,15 mg/ml and 17,90 mg/ml respectively for *Acorus calamus* and *Calendula officinalis*.

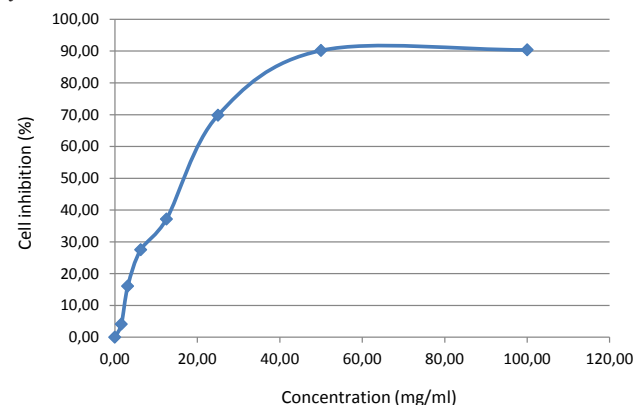


Fig 2. Anticancer effect of Calendula officinalis extract on NFS-60 cell line

Source: developed by the authors

Table 3
Anticancer activity of Calendula Officinalis against NFS-60 (suspension) mouse myeloid cell line

	Dilution	Concentration (mg/ml)	Absorbance (O.D)	Cell inhibition (%)
A	K+	100	0,054	90,36
B	1:2	50	0,055	90,18
C	1:4	25	0,169	69,82
D	1:8	12,50	0,352	37,14
E	1:16	6,25	0,406	27,50
F	1:32	3,12	0,470	16,07
G	1:64	1,56	0,537	4,11
H	K-	Cell control	0,560	0,00

Source: developed by the authors

Different extracts of the plant exhibited different activity on different cell lines. This selectivity could be due to the sensitivity of the cell line to the active compounds in the extract or to tissue specific response. The effect of the samples on the proliferation of NFS-60 was expressed as the%

Conclusion. Thus, as a result of conducted researches was established that the extracts have a different cytotoxicity on cancer cell line NFS-60. This reaction is species-specific and can be explained by the different composition of secondary metabolites in plants studied. Extract of sweet flag (*Acorus calamus*) have greater cytotoxic effect than extract of *Calendula officinalis*. These results show the prospects of using of extracts of these medicinal herbs in the treatment of cancer. The rhizome of *A. calamus* and flowers of *Calendula officinalis* might be considered as a potential sources of secondary metabolites which may be developed as precursors for anti-cancer drugs and isolation and purification of these active compounds are useful in anti-cancer drugs development.

References:

- Unnikrishnan M.C. Tumor reducing and anticarcinogenic activity of selected species / Unnikrishnan M.C., Kuttan R. // Cancer Letter. – 2009. – P. 85-89.
- Park E.J. Botanicals in cancer chemoprevention / Park E.J., Pezzuto J.M. // Cancer Metastasis Rev. – 2002. – № 21. – P. 231-255.
- Dorai T. Role of chemopreventive agents in cancer therapy / Dorai T., Aggarwal B.B. // Cancer Lett. – 2004. – № 215. – P. 129-140.
- Surh Y.J. Cancer chemoprevention with dietary phytochemicals / Surh Y.J. // Nat Rev Cancer. – 2003. – № 3. – P. 768-780.
- Divya G. Pharmacological Activities of Acorus calamus: A Review / Divya G., Gajalakshmi S., Mythili S., Sathiavelu A. // Asian Journal of Biochemical and Pharmaceutical Research. – 2011. – № 4. – P. 57-64.
- Nelia Mittal. Pharmaceutical and Biotechnological Potential of Acorus calamus Linn., An Indigenous Highly Valued Medicinal Plant Species / Nelia Mittal, Ginwal H.S., Varsliney V.K. // Phcog Rev. – 2009. – № 3. – P. 93-103.
- Raina V.K. Essential oil composition of Acorus calamus L. from the lower region of the Himalayas / Raina V.K., Srivastava S.K., Syamasunder K.V. // Flavour Fragr Jour. – 2003. – № 18. – P. 18-20.
- Kalvatchev Z. Anti-HIV activity of extracts from Calendula officinalis flowers / Kalvatchev Z., Walder R., Garzaro D. // Biomed Pharmacother. – 2007. – № 51. – P. 176-180.
- Boucaud-Maitre Y. Cytotoxic and antitumoral activity of Calendula officinalis extracts / Boucaud-Maitre Y., Algernon O., Raynaud J. // Pharmazie. – 1988. – № 43. – P. 220-221.
- Gaidhani S.N. In-vitro anticancer activity of standard extracts used in ayurveda / Gaidhani S.N., Lavekar G.S., Juvekar A.S., Sen S., Singh A., Kumari S. // Phcog Mag. – 2009. – № 5. – P. 425-429.
- Mosmann Tim. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays / Mosmann Tim // J Immunol Meth. – 1983. – № 65. – P. 55-63.
- Caputo J.L. Biosafety procedures in cell culture / Caputo J.L. // J. Tissue Cult. Methods. – 1988. – № 11. – P. 223-227.

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ОЦІНКА ПРОТИРАКОВОЇ АКТИВНОСТІ ACORUS CALAMUS L. ТА CALENDULA OFFICINALIS L. IN VITRO

Анотація

Протягом багатьох століть айр використовували у народній медицині для лікування інфекційних та неінфекційних захворювань. Фітофармакологічні дослідження різних екстрактів календули показали протизапальні, антибактеріальні, протівірусні властивості, які мають терапевтичний інтерес. Проте, дуже мало відомо про їх протираковий ефект. У даному дослідженні було оцінено протипухлинну активність водних екстрактів рослин лепехи і календули (Asteraceae) *in vitro*. Цитотоксичну активність екстрактів перевіряли за допомогою МТТ-тесту на культурі клітин мієломи миші (NFS-60).

Ключові слова: *Acorus calamus*, *Calendula Officinalis*, екстракт, протираковий, NFS-60, МТТ-тест.

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ОЦЕНКА ПРОТИВОРАКОВОЙ АКТИВНОСТИ ACORUS CALAMUS L. И CALENDULA OFFICINALIS L. IN VITRO

Аннотация

На протяжении многих веков айр использовали в народной медицине для лечения инфекционных и неинфекционных заболеваний. Фитофармакологические исследования различных экстрактов календулы показали противовоспалительные, антибактериальные, противовирусные свойства, которые имеют терапевтический интерес. Однако, очень мало известно об их противораковом эффекте. В данном исследовании было оценено противоопухолевую активность водных экстрактов растений айра и календулы (Asteraceae) *in vitro*. Цитотоксическую активность экстрактов проверяли с помощью МТТ-теста на культуре клеток миеломы мыши (NFS-60).

Ключевые слова: *Acorus calamus*, *Calendula Officinalis*, экстракт, противораковый, NFS-60, МТТ-тест.