Research Article *Open Access*

Journal of Biomedical Advancement Scientific Research

ISSN: 3069-0854

DOI: *doi.org/10.63721/25JBASR0124*

Pharmacognostic and Phytochemical Studies and Effects of Two Species of Elecampane (I. Macrophylla and I. Rhizocephala)

RA Sultonov*, SJ Yusufi, WR Rajabov and GF Navruzzoda

Hatlon State Medical University Dangara, Tajikistan
State Educational Institution "Tajik State Medical University named after Abuali Ibni Sino, Dushanbe, Tajikistan
State Institution "Scientific Research Pharmaceutical Center of the Ministry of Health and Social Protection of the Republic of Tajikistan", Tajikistan

Citation: RA Sultonov, SJ Yusufi, WR Rajabov, GF Navruzzoda (2025) Pharmacognostic and Phytochemical Studies and Effects of Two Species of Elecampane (I. Macrophylla and I. Rhizocephala). J. of Bio Adv Sci Research, 1(3):1-6. WMJ/JBASR-124

***Corresponding author:** RA Sultonov, State Educational Institution "TSMU named after. named Abuali Ibni Sino, Tajikistan.

Submitted: 14.10.2025 **Accepted:** 21.10.2025 **Published:** 03.11.2025

Keywords: Molecular Dynamics, Spin Label, Medicinal Plants, Elecampane, Hydrogen Peroxide, Electron Paramagnetic Resonance (EPR), Nitroxyl Radical, Thin-Layer Chromatography (TLC), Chromatograme Toncy (ChT)

Dushanbe

The effect of hydrogen peroxide on the molecular structure of the medicinal plant elecampane was studied using spin labeling. It was shown that the effect of hydrogen peroxide depends on its concentration. In the EPR spectra, line broadening occurs with successive increases in hydrogen peroxide concentration, meaning the signal shifts from a more disinhibited state to an inhibited state. It was established that with the growth of spectral parameters (2A' z is the distance between the outer extrema; ΔH -1 is the line width in the low field; ΔH +1 is the line width in the high field and ΔH 0 is the width of the central component of the EPR spectrum), the studied samples in the medium (elecampane root + hydrogen peroxide + nitroxyl radical) become: a more viscous solution, and with a large volume of hydrogen peroxide concentration (6.0 ml) a strong thick solution is obtained and structural transitions occur, accompanied by a noticeable increase in the rotational diffusion of the radical in the studied samples, which indicates an increase in the interaction between the molecules and the spin label.

Pharmacognozycal and Fitochimical Study of the Medicinal Plant to Inula l (I. Macrophylla I I. Rhizocephala) in Oncology Rasultonov, SJ Ysufi, Urragabov, GF Navruzzoda

The spin labeling method was used to study the effect of hydrogen peroxide on the molecular structure of the medicinal plant elecampane. It is shown that the effect of hydrogen peroxide depends on the volume of its concentration. In the EPR spectra, with a sequential increase in the volume of hydrogen peroxide concentration, the line broadens, that is, the signal from a more disinhibited signal goes to a retarded form. It was found that with an increase in the spectral parameters (2A'z is the distance between the external extrema; ΔH-1 is the line width in a low field; ΔH + 1 is the line width in a high field and ΔH0 is the width of the central component of the EPR spectrum), samples in the medium (high elecampane root + hydrogen peroxide + nitroxyl radical) becomes: a more viscous solution, and with a large volume of hydrogen peroxide concentration (6.0 ml), a strong thick solution is obtained and structural transitions occur, accompanied by a noticeable increase in the rotational diffusion of the radical in the studied samples, which indicates an increase in the interaction between molecules and the spin label.

Relevance

Medicinal plants are currently a valuable tool for the treatment and prevention of chronic diseases. At the same time, most plants have the advantage of being low in toxicity. Furthermore, medicinal plants grown in ecologically clean conditions are considered more effective [1-3]. In examining the inhibitory effects of plant organs of two species of elecampane, it should be especially noted that we have described several species of the genus Inula. L., growing on the southern slope of the Hissar Range. It was shown that of the 10 elecampane species present in the flora of Tajikistan, 6 are found in the study area.

The Aim of this Work is to study the phytochemical inhibitory effects of plant organs of two species of elecampane (I. Macrophylla and I. Rhizocephala) on cancer cells.

Material and Methods of Research

The work was carried out at the Khatlon State Medical University Department of Pharmaceutical and Toxicological Chemistry of the State Educational Institution "Tajik State Medical University named after Abuali" Ibni Sino ", State Institution "Scientific Research Pharmaceutical Center of the Ministry of Health and Social Protection of the Republic of Tajikistan.

Most species of the genus Elecampane have medicinal value and are used in the treatment of various ailments. The plants contain biologically active substances such as essential oils, organic acids, tannins, polyphenols, and other biologically active compounds. Therefore, they are widely used to treat conditions such as metabolic disorders, jaundice, hepatitis, gastric ulcers, convulsive crises, and others [4-6]. In this study, we used chemically pure nylon and lavsan fibers, as well as high-strength and medium-strength lavsan threads processed under various conditions. Fiber modifications were carried out using the method described. Two species of elecampane: large-leaved elecampane and root-headed elecampane were collected in the southern part of the Hissar region of Tajikistan (1800-2300 m above sea level). The collected materials were dried, cut, and stored for further study [7-10].

Results and Discussion

Total polyphenols were isolated from these two samples and their activity was tested against two types of cancer cells, CT 26 (colon cancer) and OVCAR -3. The number of cancer cells was counted using two methods: a) Bladesof Malassez and b) analysis and accounting of the viability of Real cells Time - Glo ™ MT. As a result, the two elecampane species showed no activity against OVCAR -3 cancer cells, but demonstrated very high activity against CT 26 cancer cells. The isolated polyphenols from elecampane macrophylla and elecampane rhizomatous may be proposed as anticancer agents against CT 26 cancer cells for future study and drug development.

Figure 1: Obtaining Polyphenol Extract

- A 70% ethanol solution was added to dry flour at a ratio of 100 g per 1 liter. Since light can activate certain chemical reactions or can destroy the molecules of the substance of great interest, the resulting ethanol suspension was shaken and incubated in the dark for two weeks;
- An example of a plant sample after obtaining an extract and centrifuging it.

Cancer Cell Viability Assay

To study the anticancer potential of plant polyphenol-containing extracts, a viability assay was conducted on the studied cell lines. After culturing 50 x 105 cells for 3 days (maximum 24 hours, 48 hours, or up to 72 hours), their growth was monitored, which depends on cell confluence in the flask, and a low-concentration extract was added. Then, by counting the cells using a Molasses chamber, we determined which extracts and which concentrations were most effective.

Figure 3: Diagram representing the protocol (result) of the cell viability test

For negative control, cell cultures were treated only with ethanol diluted in the same culture medium used to prepare extracts of different concentrations.

This experiment confirmed that the observed effects were not related to ethanol (used for extraction), but to the active molecules (i.e., polyphenols). Positive controls were treated as described above, but with M2Y N.

Cell counting: Two methods were used to count cancer cells: **Method I: Molasses chamber.** After incubating cancer cells in the culture medium and the test extract, the cells are counted after 24, 48, and 72 hours. Adherent cells must be recovered using the enzyme trypsin (to separate the cells from the substrate). To do this, the cells are incubated with trypsin for two minutes at 37 ° C, and then, to block enzymatic activity, bovine serum is added to the culture medium. Next, remove the flask containing the cells and centrifuge the entire cell pellet for 10 minutes at 1200 rpm. After centrifugation, the cell count is counted.

Method 1: For cell counting, use Molasses blades, which consist of a glass slide etched with a grid of 25 squares containing 20 smaller squares. To count cells, apply 10 µl of cell suspension to the Molasses blade and count the number of cells in 10 rectangles. The volume of a rectangle is 0.01 µl, and given 10 rectangles, the result is simply multiplied by 104 to obtain the number of cells per 1 ml (Figure 3).

The extract must be diluted 1/500 with culture medium, corresponding to the cancer cell line. The diluted extract is then added to the flank where the cells are incubated.

Incubation is carried out at 37 ° C, 5% CO2, and humidity above 80%. Cell growth is monitored for 24, 48, and 72 hours. Photographs are taken at magnification (4x, 20x, and 40x). Cell morphology is observed, and cells are first counted using a Molasses chamber.

Photo showing a 10µl cell counting mixture in a clinic B- Counting example: B-1 represents a counting rectangle where there are 25 small squares (red around the square) in that rectangle.

In Figure 2, we take a square as an example. Under a microscope, it's always clear that the square contains 17 cells. To obtain a good result, approximately ten squares are required. The cells are circled in green on the outer sides of the square. This method requires excluding the two outer sides of the square, and it was also deliberately decided not to count the cells present on the bottom and left sides (Figure 4).

Method 2: Cell Viability Assay: Real Time - Glo ™ Cell Viability. If some promising results are found after the first count, then Real-time-Glow ™ Cell should be used. Viability The MT Assay for cell viability analysis. This kit, used in the laboratory, can confirm the results obtained. The advantage of this kit is that it can be used directly on the obtained cultures, without even washing them. The kit consists of a substrate (Real-time-Glow ™ Cell Viability Substrate) ViabilityAssay MT), which can cross cell membranes without inducing damage, and an enzyme that cannot cross cell membranes. The MT cell viability substrate will be converted into NanoLuc substrate in living cells. After depletion, it can be released from the cells and maintained by luciferase. NanoLuc , where this reaction emits light (radiation), (fluorescence). Fluorescence is proportional to the number of living cells in the culture.

- Diagram showing the mode of action of the kit where dead cells cannot reduce the MT cell viability substrate, so there is no fluorescence.

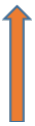
- Schematic showing the protocol (results): After starting the reaction with the Real-time-Glow ™ CellViabilityAssay MT, incubate the samples at 37 o C.

Calculating the Survival Index: After each survival test, the percentage survival of each cell line should be calculated for each extract and each dose (concentration). The percentage survival allows each extract variant to be ranked in order of effectiveness, with the extract with the lowest percentage survival being the most effective, and this, of course, depends on the cell line. Indeed, one extract variant may be more effective for one type of cancer than another.

To do this, use the following formula (number of living cells after treatment) / (5.10]^5) 5105 is the total number of cultured cells

A) Diagram showing the kit's mode of action, where dead cells cannot reduce the MT cell viability substrate, so there is no fluorescence. B) Schematic showing the protocol (results): after running the reaction with the Real-time-Glow ™ Cell Viability Assay MT, incubate the samples at 37 ° C. After each survival test, calculate the percentage of survival for each cell line according to each extract and each dose (concentration). The percentage of survival will allow each extract variant to be classified in order of effectiveness, hence the extract with the lowest percentage of survival is the most effective, and this, of course, depends on the cell line. Indeed, one extract variant may be more effective for one type of cancer than another.

To achieve TLC, a silica sheet is used as the stationary phase. A mixture of solvents will be used as the eluent. The diagram shows red and green-blue dots, which correspond to different components of the original sediment, shown in black.

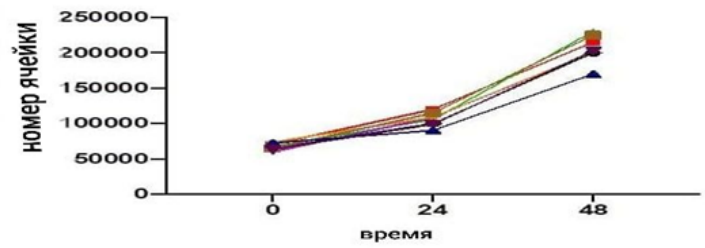


h: distance between deposit line and spot
H: the distance between the deposit line and the front line.
TLC sheets will be placed under a UV lamp to reveal the positions of the spots. The frontal ratio (Rf) is the ratio of h (the distance from the precipitation line to the spot) to H (the distance from the precipitation line to the solvent front), measured with a graduated ruler. It ranges from 0 to 1 and is characteristic of the migrating compound. The Rf for each spot is obtained from the following relationship:
Rf = (distance between deposit line and spot) / (distance between deposit line and migration front) = h / H

Once the Rf has been determined for each point on each part of each plant, the plants should be regrouped according to the migration profile of the extracts obtained from them. After polyphenol extraction, thin-layer chromatography (TLC) should be performed to identify the overall migration profile between the plants. This will make it possible to group the plants according to their migration profile. Knowing that the migration profile is characteristic of migrating compounds allows one to indirectly rank the plants by their composition.

TLC is considered a method that allows for the separation and isolation of components of a mixture. This method relies on the selective distribution of the components being separated between two phases: the mobile phase (eluent) and the stationary phase. Components are separated based on the nature of the mobile phase, the nature of the stationary phase, and the physicochemical properties of the components being separated.

The mobile phase must be soluble in the extracts to be separated, as polyphenols are non-polar molecules, so a low-polarity eluate, such as toluene, should be chosen. This way, through capillary action, the various components will migrate and distribute along the stationary phase. After air-drying , the leaves are placed under a UV lamp to reveal the location of the spots. Silica gel 60 F254 appears transparent under UV light, so any migrated compounds appear as dark spots against a light background.



Graphic representation of the effect of the alcoholic extract of elecampane polyphenols (mg/ml) on culture cells (CT26), depending on the concentration of active substances and temperature.

Graph of the effect of alcohol-soluble extracts mg/ml of elecampane on the growth of OVCAR -3 culture cells, depending on the concentration of polyphenols and temperature. The specification of the obtained results in the form of two graphs relating to the growth of cancer cells in the culture medium depending on the concentration of the alcohol extract of polyphenols mg/ml , isolated from the aboveground organs of elecampane and on temperature , clearly showed that one of the studied extracts actually strongly inhibits the growth and proliferation of Ct26 cells (Cancer CO LON) at a concentration of 0.5 and 0.25 mg /ml. However, these studied extracts at low concentrations, although not as strongly, did not inhibit the growth and proliferation of OVCAR -3 cells (Figure 10). In general, it has been shown experimentally that polyphenols isolated from the elecampane plant selectively counteract ST26 cancer cells, but with a decrease in their concentration or at low concentrations (i.e. above 0.125 mg / ml), they have little effect on their growth processes.

Thus, the obtained analysis results indicate the anti-cancer effect of elecampane polyphenols, and they can be used in cancer diseases.

About the Authors

Yusufi Salomiddin Jabbor - Tajik State Medical University named after Abu Ali ibn Sino, Doctor of Pharmaceutical Sciences, Professor, Academician of the National Academy of Sciences of Tajikistan. Address: 734003, Republic of Tajikistan, Dushanbe, Rudaki Avenue 139.

Sultonov Raufjon Azizkulovich - Khatlon State Medical University, PhD in Pharmaceutical Sciences, Associate Professor, Head of the Department of Biochemistry and Medical Biology. Address: 734003, Republic of Tajikistan, Dangara , Ismat Sharif Avenue 3. Phone: 93-919-07-44. E- mail: raufsultonov@mail.ru

Radjabov Umarali Radzhabovich – Tajik State Medical University named after Abu Ali ibn Sino, professor of the Department of Pharmaceutical and Toxicological Chemistry, Doctor of Chemical Sciences, professor Address: 734003, Republic of Tajikistan, Dushanbe. Rudaki Avenue 139. Phone: (+992) 907-46-48-29. E- mail: umarali55@mail.ru

Navruzova Ganjina Furkat - Tajik State Medical University named after Abu Ali ibn Sino, Head of the Department of Pharmaceutical and Toxicological Chemistry, Candidate of Pharmaceutical Sciences, Associate Professor. Address: 734025, Republic of Tajikistan, Dushanbe, Rudaki Avenue, 139. E - mail: ganga-tj@mail.ru

Information about the authors: Ysufi Salomiddin Djaborovich- Tajik State Medical University named after. Abuali ibn Sino Doctor of pharmasii Sciences, Professor.academic NAST Address: 734003, Republic of Tajikistan, Dushanbe. Prospect Rudaki 139.

Sultonov Raufjon Azizkulovich - Tajik State Medical University. Abuali ibn Sino, Candidate of Pharmaceutical Sciences of the Department of Pharmaceutical and Toxicological Chemistry. Address: 734003, Republic of Tajikistan, Dushanbe. Prospect Rudaki 139. Phone: 93-919-07-44. E-mail: raufsultonov@mail.ru

Radzhabov Umarali Radzhabovich - Tajik State Medical University named after. Abuali ibn Sino, Head of the Department of Pharmaceutical and Toxicological Chemistry, Doctor of Chemical Sciences, Professor. Address: 734003, Republic of Tajikistan, Dushanbe. Prospect Rudaki 139.

Telephone: (+992) 907-46-48-29. E-mail: umarali55@mail.ru

Navruzzoda Gandjina Furkat - Tajik State Medical University named after. Abuali ibn Sino, Candidate of Pharmaceutical Sciences, Associate, Address: 734003, Republic of Tajikistan, Dushanbe. Prospect Rudaki 139.

References

1. Khadzhimatov M (1989) Wild medicinal plants of Tajikistan [Text] / M.Khadzhimatov . - Dushanbe: Maorif 288-290.
2. Flora of the Tajik SSR (1986) – L.: Nauka, T.9. 434.
3. Phytochemical and pharmacological properties of medicinal plants from Uzbekistan (2017) Journal of Medicinal Plants 5: 76-82.
4. Shagiakhmetov, Yu S (2000) Pharmacological characteristics of preparations from plants of the genus Inula and their use in veterinary medicine: Abstract of a doctor of veterinary sciences dissertation / Yu.S.Shagiakhmetov . – Troitsk 35.
5. Shukurov, A.Sh (1972) The main useful wild plants of the flora of Tajikistan [Text] / A.Sh. Shukurov, N.K. Eremina , K.V. Stanyukovich // Proceedings of the Academy of Sciences of the Tajik SSR. T.15, No. 10: 62-66
6. Sultonov RA, Radzhabov UR, Yusufi SJ, Rahimi F, Navruzoda GF, et al. (2023) Study and influence of environmental factors on rotational mobility of spin-labeled medicinal plant Ferula foetida (FERULA ASS – FOETIDA L) Science and innovation 1: 87-79.
7. Sultonov RA, Radzhabov UR, Yusufi SJ, Rahimi F, Navruzoda GF, et al. (2023) An EPR study of the antioxidant properties of flavonoids, propolis, and mumijo. Science and Innovation 1: 75-79.
8. Sultonov RA, Radzhabov UR, Yusufi SD, Navruzoda GF, Yusufov I Kh (2023) Study of the molecular structure of elecampane depending on the concentration of hydrogen peroxide using the spin labeling method Science and innovation 1: 62-66
9. Radzhabov UR, Sultonov RA, Yusufi S Dzh, Yusupov I Kh, Khaidarov K Kh (2017) Synthesis and biological properties of zincas and its study by the spin labeling method. Bulletin of the Academy of Sciences of the Republic of Tatarstan 4: 97-106.
10. Rajabov UR, Sultonov RA, Yusufi SD, Yusupov I Kh, Khaidarov K Kh (2018) Antioxidant effect of iron (II) with acetylcysteine and its study by the spin labeling method. DAN RT, 61: 788-932.