Evaluation of Anticancer Action of *Martynia annua* Linn Root Extract on Different Human Cancer Cell Lines

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Authors’ contributions

This work was carried out in collaboration between both authors. Author DKG during PhD. Author RKG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and completed analysis of the study and Author RBR was supervisor of the scholar. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i26A31474

Editor(s):
(1) Dr. Vasudevan Mani, Qassim University, Saudi Arabia.

Reviewer(s):
(1) R. Senthil Kumar, Swamy Vivekananda College Of Pharmacy, India.
(2) Dario Siniscalco, University of Campania, Italy.

Complete Peer review History: http://www.sdiarticle4.com/review-history/65454

Received 16 December 2020
Accepted 21 February 2021
Published 27 April 2021

ABSTRACT

**Background:** In the last few decades, plants have been playing a vital role in treating cancer and infectious diseases. Natural products have been rediscovered as effective methods of drug production amid advances in combinatorial chemistry. Roots of *Martynia annua* are being used as a folklore remedy for the treatment of cancer and rheumatism successfully.

**Aims of the Study:** In the present study, ethanolic, aqueous and hydro-ethanolic root extracts of *Martynia annua* were screened for *in vitro* cytotoxicity activity using different cell lines.

**Settings and Design:** In the experiment, lung cancer cell lines (A549), leukemia cancer cell lines (K562), oral cancer cell lines (SCC-40), breast cancer cell lines (MCF-7) & cervix cancer cell lines (SiHa) were studied on the extracts.

**Materials and Methods:** The method used was Sulforhodamine B (SRB) assay technique in which growth inhibition of 50% (GI50) was analyzed by comparing it with standard drug Adriamycin (ADR) (doxorubicin).

**Results:** Aqueous & ethanolic extract of *Martynia annua* root had shown high anticancer activity with GI50 value 11.3µg/ml and 20.4µg/ml respectively on human leukemia cell line K-562 but for human breast cancer cell line MCF-7, human lung cancer cell line A-549, human squamous cell

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carcinoma SCC-40 and human cervical cancer cell line SiHa the extracts showed activity in more than 80µg/ml.

Conclusion: The anticancer activity of aqueous extract of *Martynia annua* root was found superior than the ethanolic extract in Human Leukemia Cell Line K-562. The study indicates that the *Martynia annua* root extracts are most effective against the fast proliferative cells (Leukemic cells) and possibly a cell cycle arrest (needed to be proved as future perspective) is the mode of action of the extract. To study its effect on targeted cancers, specific in vivo scientific studies and clinical trials should be carried out by further researchers.

Keywords: *Martynia annua*; kakanasika, lung cancer cell lines (A549); leukemia cancer cell lines (K562); oral cancer cell lines (SCC-40); breast cancer cell lines (MCF-7); cervix cancer cell lines (SiHa); Anticancer activity, sulforhodamine B assay method.

1. INTRODUCTION

Cancer has a major impact on society across the world due to its high morbidity and mortality [1]. Noncommunicable diseases (NCDs) accounted for 71 percent of total fatalities worldwide. NCDs are estimated to account for 63 percent of all deaths in India, and cancer has been one of the leading causes of cancer (9 percent). The cases of lung cancer are more in men than women. For men out of all cancer cases it is 2nd most common type & for men and women both it is 5th most common type of cancer. The cases of lung cancer are more in men than women. For men out of all cancer cases it is 2nd most common type & for men and women both it is 5th most common type of cancer. Leukemias being the most common and lymphomas are also frequently occurring childhood cancer in most industrialized and also in developing countries [2,3]. Oral cancer accounts for nearly 30% of all cancers in India [4]. Breast cancer is the most common cancer in women worldwide, while cervical cancer is the third most common cancer of women after breast cancer [5]. Current approaches to combat cancer rely primarily on chemotherapy and radiation, although these therapies have saved numerous lives of various cancer sufferers but themselves carcinogenic and causative agents for recurrence and increase the chance to develop metastatic disease. Hence, most of the research work on cancer drugs is targeted on plants and plants derived natural products.

Two renowned Ayurvedic Treatise i.e. Charaka [6] & Sushruta Samhitas [7] and according to these Samhitas cancer is described as non-inflammatory or inflammatory growth and mentioned either Arbuda (large neoplasm) or Granthi (small neoplasm). Three-body management systems, viz., Lymph System (Kapha or water) Venous System (Pitta or fire), & Nervous System (Vata or air) are mentioned in Ayurvedic literature and a balanced coordination of Tridosha are essential for normal healthy body. All or any of the uncontrolled dosha may lead to the development of neoplasm that may be benign or malignant depending upon the severity of the incoordination [7].

According to the principles of Ayurveda, no disease can be named on its own because it varies from person to person in its clinical manifestation, illness & in medication [8]. Therefore, according to Ayurveda, cancer is pictured as a tridoshic disease that can spread because of the interaction of vitiated vata, pitta, and kapha. Agni or Pitta responsible for digestion is present in every cell of body. An inverse relationship between tissue growth & the agni present in the tissue is a reason behind the unnatural tissue grown (Arbuda) on decreasing Dhatwagni (deranged metabolism). Concept of metastasis is different in Ayurveda from modern allopathy. As per Ayurveda, metastasis is caused by the active Vata dosha. Vata may be associated with anabolic growth process where kapha to the anabolic phase. Cancer develops due to the imbalance of metabolism where vata aggravates and kapha gets suppressed. Kapha being heavy and gross is responsible for the abnormal growth of the malignant tumour forming cells.

For thousands of years, plants have been a good source of medicine to treat ailment and maintain health. Mostly roots, flowers, leaves, root, stem, barks and seeds of plants are rich in secondary metabolites that produce definite pharmacological effects on human body.

*Martynia annua* (L.) belongs to family Martyniaceae is an herbaceous annual plant, found throughout India. Its excellent dispersal mechanism has helped it spread throughout the tropical world as a weed [9].
The phytochemical analysis showed *Martynia annua* root having many medicinally important secondary metabolite like carbohydrate, Protein, Alkaloid, Cardiac glycoside, Flavonoids, Tannins, Anthraquinone glycoside, Steroids and Triterpenoids, which indicates that the plant root possesses high profile therapeutic values [10].

Roots of *Martynia annua* are used as tonic and in vitro antioxidant activity study revealed that plant root is potent antioxidant drug [11]. In Ayurveda, the plant is known as *kakanasika*, which is being used to treat in various disorders [12]. In Tribal Pockets of Satpura Plateau in Madhya Pradesh, Root paste of *M. annua* is used to treat Cancer and rheumatism [13].

Literary study revealed that this plant root is most useful part [13] and no evidence found for anticancer activity through in vitro cell line. Therefore, there was a need of scientific validation of anticancer activity study of *Martynia annua* root.

### 2. MATERIALS AND METHODS

#### 2.1 Plant Collection

The roots of *M. annua* were collected from Campus of Govt. Ayurved College, Gwalior (Madhyapradesh) during the month of January 2018 and it was authenticated from Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore (Karnataka). Its authentication voucher number is SMPU/RARIMD/BNG/2017-18, Bengaluru, Dated 26/02/2018.

#### 2.1.1 Plant extraction

The dried *M. annua* root was ground and extracted with Water, Ethanol and Hydro-ethanol using Soxhlet apparatus. All the three extracts obtained are stored at 2-4°C for further uses for the study.

#### 2.2 Anticancer Activity

##### 2.2.1 Cell lines

Five human cancerous cell lines of Lung(A-549), Leukemia(K-562), Oral(SCC-40), Breast(MCF-7) and Cervix (SiHa) were selected to assess anticancer activity of *Martynia annua* roots extracts and the study was completed in Tata Memorial Centre – Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai, India.

##### 2.2.2 Equipments and chemicals

SRB colorimeter, 25 cm² tissue culture flasks, 15 ml centrifuge tubes, 96 well plate, CO₂ incubator, Haemocytometer, Drug dispensing machine, Multi-channel automated pipette, Elisa reader
etc. were used for in vitro study. The chemicals like liquid nitrogen, DMSO, SRB dye, 1% acetic acid Ethanol, 10%TCA were used in the current study.

2.3 Preparation of Cell-Material (Human Cancer Cell Lines culture) [14–16]

The selected cell lines were grown in cell culture flasks containing RPMI 1640 medium. The cell culture flask was then kept in a CO₂ incubator at 37.5°C for 24-48 hrs for cell division. Cell count was adjusted according to the titration readings (approximately 1x 10⁵ cells/ml) Cell culture was done under all aseptic conditions.

2.3.1 Determination of the anticancer activity of root extract of Martynia annua on selected human cancer cell lines by using in vitro SRB assay

The sulforhodamine B (SRB) assay method is a rapid, sensitive, reliable method for analyzing the cellular protein content of adherent and suspension cultures in 96-well microtiter plates. It is an ideal method for ordinary laboratory purposes and large-scale applications as in disease-oriented studies in which in vitro anticancer drug discovery screening is to be undertaken. It gives a colorimetric end that is nondestructive, indefinitely stable and visible to the unaided eye. It gives a sensitive measure of drug-induced cytotoxicity is valuable in quantitating clonogenicity and is appropriate to high volume, automated drug screening [17]. In present study experiment was performed in triplicates.

2.3.2 Preparation of solution of test drug [14]

The test substance name and container were confirmed before preparing the drug solution. The molecular weight of the study drug was not known and hence, the solution was prepared in a proportion of 1:100 (stock solution: star solution). The same concentration was used for the vehicle control as well as for positive control. The solution was made using ratio 1:100 in a test tube and it was mixed gently with the help of a magnetic stirrer. The experimental drugs were solubilized in a solvent at 400-fold the desired final maximum test concentration and stored frozen before use. At the time of experimental drug addition, an aliquot of frozen concentrate was unfrozen & diluted to ten times the desired final maximum test concentration with a complete medium containing test drug at a concentration of 10 µg/ml, 20 µg/ml, 40 µg/ml and 80µg/ml.

2.3.3 Plate preparation and drug addition [14,18]

For in vitro anticancer screening, 100 µl cells/well were seeded into 96-well micro titer plates so that every well receives 5X10⁵ cells. After cell inoculation, the plates were incubated at 37°C, 5% CO₂, 95% air, and 100% relative humidity for 24h. The incubated plates were then placed in a drug dispensing machine inside the laminar flow hood to avoid bacterial contamination. The study drug (Aqueous extract (AMA), Ethanolic Extract (EMA), and Hydro-ethanolic extract (HEMA) of Martynia annua roots) were tested in 96 well plates with its 4 dilutions (4 different concentrations) at 10, 20, 40, 80 µg/ml in triplicates. The Adriamycin (ADR) (doxorubicin) was used as a positive control drug for comparative screening.

2.3.4 In vitro anticancer screening by Sulforhodamine B (SRB) assay method [18,19]

In addition to the drug, the plates were incubated further for 48 hours at 37°C in a humidified CO₂ (5%) incubator. After incubation, 50µL of 30% TCA was added to fix the cells to the bottom of the wells; After 60 minute’s incubation at 4°C, plates were washed gently under tap water and were air-dried at room temperature. Then 100 µl SRB (Sulfurhodamine B) reagents were added into each well and left for 15 minutes and the SRB dye was removed by washing the plates with tap water. 1% of acetic acid was used to remove unbound SRB dye. After air-drying, 0.1ml of 10mM TRIS buffer was added and the absorbance was read on the Elisa plate reader at the wavelength of 540 nm to 690 nm. The optical density of drug-treated cells was compared with that of control cells and growth inhibition was calculated as percent values.

2.4 Endpoint Measurement [14]

Using the six absorbance estimations (time zero (Tz), control growth (C) and the test growth at the four-drug concentration levels (Ti)), the percentage of growth was calculated at each of the test drug concentration levels. The percentage of growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent of growth was expressed as the...
ratio of average absorbance of the test well to the average absorbance of control wells. Percentage growth inhibition was calculated as \[\frac{[(T_i - T_z) / C - T_z]}{100}\] for concentration for which \(T_i > T_z\) (Ti-Tz) positive or zero and \[\frac{[(T_i - T_z) / T_z]}{100}\] for concentration for which \(T_i < T_z\). (Ti-Tz) negative. The dose-response parameter was calculated for each test drug. Growth inhibition of 50% (GI\textsubscript{50}) was calculated from \[\frac{[(T_i - T_z) / C - T_z]}{100}= 50\] The concentration resulting in total growth inhibition (TGI) was calculated from \(T_i = T_z\). The LC\textsubscript{50} was calculated from \[\frac{[(T_i - T_z) / T_z]}{100}= -50\]. Values were calculated for each of these three parameters. The values were expressed as greater or less than the maximum or minimum concentration tested. From the values, the graph was plotted and the results were given in terms of LC\textsubscript{50}, TGI and GI\textsubscript{50} values.

3. RESULTS AND DISCUSSION

The results on percentage control growth of Martynia annua root extracts on MCF-7, K-562, A-549, SCC-40 7 SiHa human cancer cell lines are shown in Tables 2 to 6 and growth curve graph are shown in Graph 1 to 5 accordingly.

\[
\text{LC}_{50} : \text{Concentration of drug resulting in a half reduction in the measured protein at the end of the drug treatment as related to that at the beginning indicating a net loss of fifty percent cells following treatment.}
\]

\[
\text{GI}_{50} : \text{Growth inhibition of fifty percent (GI50) resulting in a fifty percent reduction in the net protein increase.}
\]

\[
\text{TGI} : \text{Drug concentration resulting in total growth inhibition (TGI)}
\]

\[
\text{NE} : \text{Non- evaluable data. Experiment needs to be repeated using different set of drug concentrations,}
\]

\[
\text{AMA} : \text{Aqueous extract of M. annua Root,}
\]

\[
\text{HEMA} : \text{Hydro-ethanolic extract of M. annua Root,}
\]

\[
\text{EMA} : \text{Ethanolic extract of M. annua Root,}
\]

\[
\text{ADR} : \text{Adriamycin (ADR) (doxorubicin)}
\]

The plant Martynia annua is being used as a folklore remedy for the treatment of rheumatism and cancer etc successfully. Preclinical and clinical research are essential to evaluate integrate new herbal drugs into clinical routine. \textit{In-vitro} (mechanism based on) screening of herbal medicine is mandatory in the initial phases of plant drug research before taking them to \textit{in-vivo} study to evaluate their efficacy [20].

Cancer develops when the balance between cell proliferation and cell death is disturbed, and aberrant cell proliferation leads to tumor growth. In this study, the anticancer activities of all three root Extracts of \textit{M. annua} roots were investigated using a Sulfurhodamine B assay on 5 selected human cancer cell lines (Lung Cancer Cell Lines (A549), Leukemia Cancer Cell Lines (K562), Oral Cancer Cell Lines (SCC-40), Breast Cancer Cell Lines (MCF-7) and Cervix Cancer Cell Lines (SiHa)) because it is a well-established \textit{in vitro} method for cytotoxicity against cancer cell lines and here it was utilized to determine the selective activity of the potential anticancer drug.

Amongst currently available methods for advanced \textit{in vitro} screening techniques are SRB, MTT, clonogenic, fluorescent assays & dye exclusion test. SRB assay is particularly useful for qualitative analysis. It provides better linearity with cell numbers than MTT assay and is also highly sensitive [21]. It is also reported that plant extracts interfere with the MTT tetrazolium assay and may lead to false-positive results [22]. Hence, it is a more appropriate assay for screening due to its accuracy and feasibility. The anti-proliferative SRB assay was performed to assess growth inhibition [19, 23–28].

To assess the anticancer activity of roots of Martynia annua a cell line-based anticancer activity assay has been designed as above. In the design, the cell line selection is done to cover the diversified cell types based on their specific physiological character that may affect their rate of proliferation, cell permeability and hormone-dependent growth.

1. Human Breast cancer cell line and cervical cancer cell line: These are specific female organ cell lines and their cell division is very much female hormone-dependent.
2. Leukemia cell line: These are the cells of the hemopoietic system and these cells are highly proliferative cell lines.
3. Human lung cancer cell line: Lung is a tissue with high gaseous exchange capacity, very permeable to gases and these tissues are high blood perfused in the body.
4. Squamous cell line: These are flat epithelial cell lines that form components of the vascular system; exchange of nutrients and wastes occurs across these thin cells.
The three extracts (Aqueous, Hydro-ethanolic and Ethanolic) were developed to extract the compounds of high polarity like salts of alkaloids, phenolics, etc (Aqueous Extract) comparatively less Polar organic molecules like biological amines, glycosides, etc (ethanolic extract) and compounds of intermediate polarity (Hydro-ethanolic extract) to get a wide variety of components in the extracts.

These extracts have been subjected for their anticancer activity evaluation on all the above five cancer cell lines and evaluated for the three crucial parameters i.e. LC$_{50}$, TGI & GI$_{50}$. Adriamycin (ADR) (doxorubicin) has been used as a standard drug and four-drug dose levels 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml has been used in the study. The findings of the experiments can be summarized as follows.

The study of the activity table showed that the Aqueous extract is highly active against the Leukemia cell lines and its GI$_{50}$ is 11.3µg/ml. The ethanolic extract is also active but with GI$_{50}$ 20.4 µg/ml.

**Growth curves of different Human cancer cell lines**

| Drug concentrations (µg/ml) calculated from graph |
|-----------------------------------------------|
| **MCF-7** | LC$_{50}$ | TGI | GI$_{50}^*$ |
| AMA | NE | NE | >80 |
| HEMA | NE | NE | >80 |
| EMA | NE | NE | >80 |
| ADR | NE | <10 | <10 |

Graph 1. Human breast cancer cell line MCF-7

| Drug concentrations (µg/ml) calculated from graph |
|-----------------------------------------------|
| **K-562** | LC$_{50}$ | TGI | GI$_{50}^*$ |
| AMA | NE | NE | 11.3 |
| HEMA | NE | NE | NE |
| EMA | NE | NE | 20.4 |
| ADR | NE | <10 | <10 |

Graph 2. Human leukemia cell line K-562
Drug concentrations (µg/ml) calculated from graph

|     | LC50 | TGI | GI50* |
|-----|------|-----|-------|
| AMA | NE   | NE  | >80   |
| HEMA| NE   | NE  | >80   |
| EMA | NE   | NE  | >80   |
| ADR | NE   | NE  | <10   |

Graph 3. Human lung cancer cell line A-549

Drug concentrations (µg/ml) calculated from graph

|     | LC50 | TGI | GI50* |
|-----|------|-----|-------|
| AMA | NE   | NE  | >80   |
| HEMA| NE   | NE  | >80   |
| EMA | NE   | NE  | >80   |
| ADR | <10  | <10 |       |

Graph 4. Human squamous cell carcinoma SCC-40
Drug concentrations (µg/ml) calculated from graph

| SiHa | LC50 | TGI | GI50* |
|------|------|-----|-------|
| AMA  | NE   | NE  | >80   |
| HEMA | NE   | NE  | >80   |
| EMA  | NE   | NE  | >80   |
| ADR  | NE   | <10 | <10   |

Graph 5. Human cervical cancer cell line SiHa
All the three extracts when subjected for their activity against the other four cell lines the GI_{50} value was found more than 80µg/ml as shown in the table and the graph.

Anthraquinones & Terpenoids chemical classes present in extract may be mainly responsible for anticancer effect on all five cancer cells (Leukemia, Lung A-549 & SCC-40, Breast & cervical.)

Steroids chemical class present in extract along with above two classes may additionally produce anticancer effect on hormone dependent cancer cells (Breast & cervical)

The study indicates that the *Martynia annua* root extracts are highly effective against the fast proliferative cells (Leukemic cells) and possibly a cell cycle arrest (needed to be proved as future perspective) is the mode of action of the extract.

*Martynia annua* root have Kashaya rasa, Madhura Anurasa, Unshna Snigdha Guna Katu Vipaka and Ushna Virya [29], tridoshashamak, deepan, amapachana & raktaarbudnashan properties.

These properties of dravya help to breakdown the pathogenesis of the disease as shown in flow diagram.

### 3.1 Effect on Dosha

*Martynia annua* root encounters pitta dosha by virtue of its Kashaya rasa, madhura rasa respectively and kapha dosha by virtue of its Unshna Snigdha Guna Katu Vipaka, Ushna Virya and vata dosha by virtue of its madhura rasa Snigdha Guna, Ushna Virya Therefore, it acts as tridoshhara.

### 3.2 Effect on Dushya, Agni and Ama

*Martynia annua* root is deepana, pachana due to its Ushna Guna Katu Vipaka Ushna veerya So, it encounter agnimandya & potentiates the weakened dhatwagni and help in amapachana thereby alleviate dushit rasa rakta and mansa dhatu.

### 3.3 Effect on Srotas

Due to amapachana and maargaan Vivrunoti action of Unshna Guna Katu Vipaka Ushna veerya, all the involved channels are dilated. So the Srotorodha is removed and rasa- rakta-mansavaha srotovishodhana occurs.

### 3.4 Effect on Vyadhi

present study results revealed that *Martynia annua* root is potent anticancer as well as antioxidant drug and due to its rasayana & raktaarbudnashan karma it will be effective in the management of Leukemia (Blood cancer).

In nut shell *Martynia annua* root have Kashaya rasa, Madhura Anurasa, Unshna Snigdha Guna Katu Vipaka and Ushna Virya, tridoshashamak, deepan, amapachana & Raktaarbudnashan properties. These properties of dravya help to breakdown the pathogenesis of the disease.
Table 1. % Control growth of human breast cancer cell line MCF-7

| Drug concentrations (µg/ml) | Experiment 1 | Experiment 2 | Experiment 3 | Average Values |
|-----------------------------|--------------|--------------|--------------|----------------|
| AMA                         | 10.00        | 20.00        | 40.00        | 80.00          |
| HEMA                        | 101.4        | 101.6        | 129.2        | 163.1          |
| EMA                         | 130.9        | 139.2        | 157.0        | 184.5          |
| ADR                         | 19.5         | 5.7          | -0.5         | -4.2           |

Table 2. % Control growth of human leukemia cell line K-562

| Drug concentrations (µg/ml) | Experiment 1 | Experiment 2 | Experiment 3 | Average Values |
|-----------------------------|--------------|--------------|--------------|----------------|
| AMA                         | 50.3         | 42.2         | 81.0         | 108.2          |
| HEMA                        | 53.5         | 65.1         | 124.5        | 180.0          |
| EMA                         | 54.4         | 63.6         | 39.1         | 80.7           |
| ADR                         | 43.6         | 42.4         | 39.8         | 39.6           |

Table 3. % Control growth of human lung cancer Cell Line A-549

| Drug concentrations (µg/ml) | Experiment 1 | Experiment 2 | Experiment 3 | Average Values |
|-----------------------------|--------------|--------------|--------------|----------------|
| AMA                         | 109.1        | 93.8         | 90.2         | 94.5           |
| HEMA                        | 97.0         | 89.3         | 95.3         | 93.0           |
| EMA                         | 96.7         | 90.6         | 90.0         | 80.6           |
| ADR                         | 1.9          | 1.2          | 0.7          | 1.0            |
### Table 4. % Control growth of human squamous cell carcinoma SCC-40

| Drug concentrations (µg/ml) | Experiment 1 | Experiment 2 | Experiment 3 | Average Values |
|----------------------------|-------------|-------------|-------------|---------------|
| AMA                        | 118.6       | 112.7       | 106.2       | 110.6         |
| HEMA                       | 128.5       | 113.4       | 110.9       | 114.5         |
| EMA                        | 125.8       | 114.8       | 109.4       | 111.4         |
| ADR                        | -59.4       | -58.0       | -69.0       | -66.4         |

### Table 5. % Control growth of human cervical cancer cell line SiHa

| Drug concentrations (µg/ml) | Experiment 1 | Experiment 2 | Experiment 3 | Average Values |
|----------------------------|-------------|-------------|-------------|---------------|
| AMA                        | 110.7       | 120.3       | 125.8       | 125.9         |
| HEMA                       | 115.5       | 118.8       | 116.5       | 117.3         |
| EMA                        | 103.4       | 108.2       | 105.5       | 103.5         |
| ADR                        | -42.8       | -40.1       | -38.6       | -44.3         |
The evaluation of possible mode of action thus seems to be linked with the cell cycle as some well-known anticancer drugs isolated from plants like Vincristine and Vinblastin and paclitaxel act on the cell cycle cause cell cycle arrest and thus, exhibit their anticancer activity [30].

4. CONCLUSION

In the present in-vitro anticancer study, a sincere attempt has been made to conclude. According to SRB assay protocol, the aqueous & ethanolic extract of Martynia annua root had shown high anticancer activity with GI\textsubscript{50} value 11.3µg/ml and 20.4µg/ml respectively on Human Leukemia Cell Line K-562 but for Human Breast Cancer Cell Line MCF-7, Human Lung Cancer Cell Line A-549, Human Squamous Cell Carcinoma SCC-40 and Human Cervical Cancer Cell Line SiHa, the extract showed activity in more than 80µg/ml. In present study Aqueous extract was found superior than ethanolic extract in Human Leukemia Cell Line K-562 and Martynia annua root extracts are most effective against the fast proliferative cells (Leukemic cells).

The growth inhibition of these cells are possible not only by direct cytotoxicity but also by competitive inhibitors for hormonal receptors (Breast MCF-7 & Cervical Cancer Cell Line SiHa), differentiating these cell lines to the other three selected cell lines (Leukemia, Lung A-549 & SCC-40).

Anticancer activity of Martynia annua Linn is also justifiable on the basis of Rasapanchak.

Possible cell cycle arrest that is the mode of action of many anthracycline anticancer drugs that by either inhibiting DNA replication & by intercalation or terpenoid like paclitaxel which inhibit the disassembly of microtubules and arrest the mitotic phase. (Anthaquinones and Terpenoids both showed their presence in the chemical screening of the above drugs).

This hypothesis needed to be proved by further experimentation.

To study its effect on targeted cancers, specific in vivo scientific studies and clinical trials should be carried out by further researchers.

5. LIMITATIONS

Compounds may show negative results for a prodrug(cyclophosphamide, capecitabine, etc) which gets activated after body metabolism or vice versa [31,32]. for example the Cyclophosphamide requires to be activated to its active form by liver enzymes to show its anticancer activity. In absence of these enzymes, the actual cytotoxic activity of cyclophosphamide cannot be assayed by a cell line study [33].

In the current research, we have examined the effect of our research drug in-vitro only but in clinical practice, the effect of any drug is dependent on complete knowledge of roga and rogi as described by Acharya Vagbhhta that specificity of each of ten items of Dashavidha pareeksha (Dooshyam, Desam, Balam, Kalam, Analam, Prakruti, Vyayas, Satwam, Satmyam, Aaharam) and the manner in which they are required to be examined is essential for a successful treatment plan [34].

5.1 The Innovation of Current Research

Based on the Folklore database Martynia annua plant root is evaluated for its anticancer effect in 5 selected cancer lines. The study revealed the positive efficacy of the study drug in Leukemia (Blood cancer) originated by the K-562 cancer cell line. The current study will be a pioneer research source for further anticancer research on folklore plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

IEC was taken prior to performed study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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