EXAMINE ANTICANCER REAGENTS OF IRIDACEAE PLANT ON HUMAN TUMOR CELLS

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ABSTRACT

Cancer is a disease that affects the human population worldwide. New medicines to treat and prevent this life-threatening condition are always in demand. Natural-derived substances are attracting scientific and academic attention since they are thought to have fewer hazardous side effects than conventional therapies like chemotherapy. The effects of plant extracts on human tumour cells (MCF-7, HepG2, HCT116, HeLa, HL-60, and HaCaT) have been investigated in this paper. Crocus sativus (stigma, flowers, leaves, and corms), Juno bucharica (leaves), Gladiolus hybrid Zefir (leaves), and Iris hungarica (rhizomes) were among the Iridaceae species used in the study. All of the extracts tested were able to kill tumour cell lines in vitro in a dosage and time-dependent manner. Their tumor-cell-killing action was tissue-specific. The HCT116 colon adenocarcinoma cell line and the HL-60 acute promyelocytic leukaemia cell line were shown to be the most susceptible to treatment with the plant extracts investigated. The extracts were shown to be ineffective against HaCaT line normal human epidermal keratinocytes. In vitro, gladiolus leaf extract has very potent anticancer effects.

Keywords: Anti-cancer activity; Iridaceae; dry extracts; leaves; Crocus

I. INTRODUCTION

In both industrialised and developing countries, cancer is a serious public health concern. The ability of natural and synthetic or biological and chemical agents to reverse, inhibit, or halt carcinogenic development is known as anticancer activity. Several synthetic medications are used to treat the condition, but they have side effects, thus researchers are looking into plant-derived chemotherapeutic drugs. Ayurveda, a traditional Indian medical system based on plant medications, has proven effective in employing these natural remedies to prevent or inhibit malignant tumours using diverse lines of treatment since ancient times. People of numerous ethnic groups inhabiting various terrains in India have their own own culture, religious traditions, culinary habits, and a wealth of traditional medicinal expertise. They use herbal medicine to treat a wide range of ailments. For thousands of years, natural goods, particularly plants, have been utilised to cure a variety of ailments. From ancient times, terrestrial plants have been employed as medicines in Egypt, China, India, and Greece, and a large number of contemporary pharmaceuticals have been produced from them. Around 2600 BC, the Sumerians and Akkaidians wrote the first written documents on the medical applications of plants.

Cancer is a term used to describe a group of illnesses that are caused by a breakdown of cell cycle regulation. Cancer is linked to unregulated, aberrant cell proliferation. External causes (tobacco, chemicals, radiation, and infectious organisms) as well as internal factors cause cancer (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Cancer is a major global health issue, owing to a lack of broad and thorough early detection tools, the poor prognosis of people detected at later stages of the disease, and the disease's rising global occurrence. Cancer is, without a doubt, one of humanity's most difficult difficulties.

II. MEDICINAL PLANT DEMANDS

Plant-based medications have had a lot of success in clinical trials, so they're a popular choice for clinical

research. They are in high demand due to their non-toxic effects on normal cells and cytotoxic effects on cancer cells. Many of the species studied are from impoverished nations in Africa and Asia, where herbal remedies are common and medicinal plants are used as first-line treatments. In 2007, the World Health Organization projected that the market for plant-derived pharmaceuticals was worth $100 billion. By 2050, the commerce is estimated to be worth $5 trillion.

In developing nations, there is a significant demand for medicinal herbs, placing strain on plant populations. For informal commerce, several medicinal plants are produced from wild populations, but this cultivation is not controlled. The conservation of medicinal plants is becoming an issue that has to be addressed as a result of fast population expansion, deforestation, and expanding urbanisation. High-value medicinal plants are at danger of extinction if over-exploitation continues due to rising demand. It is critical to protect these plants. Only certain portions of wild medicinal plants are utilised in therapy, such as the bark of a tree or the bulbs and tubers of bulbous and tuberous plants. Only extracting portions of a plant can harm it and lower its chances of survival. To ensure the long-term viability of medicinal plants in underdeveloped nations, all plant parts should be included in the therapy, including the stem, leaf, root, and bark. Other conservation approaches include germplasm preservation, conserving viable seeds, cryopreservation, preserving biological material in liquid nitrogen, and tissue culture, which propagates plants in sterile conditions and may swiftly generate mature plant clones of rare species. In developed nations, these preservation procedures will also enable for industrial use.

Foods having therapeutic characteristics, such as cruciferous vegetables and berry berries, are attracting attention. Raw industrial by-products might be used to extract anticancer drugs from sources that contain these compounds. Grapes (Vitris vinifera), for example, are one of the most widely farmed crops in the world, and 'grape seed extract' is frequently used in food items owing to its human health advantages. Grape stems are a raw by-product of winemaking in the winery business. The atmosphere around the winery may be acidic as a result of the high organic load. However, because of its high polyphenolic content, it may be useful in the development of anticancer drugs and as part of a profitable programme to address environmental concerns. Grape stem extracts have been proven to exhibit antioxidant characteristics, protect DNA from reactive oxygen species, and have anti-carcinogenic potential in a variety of cancer cell lines, including cervical cancer, thyroid cancer, and others.

III. MATERIALS AND METHODS

- **Plant material**

In June 2020, botanical gardens obtained Juno bucharica (Foster) Vved. leaves (a voucher specimen CWU0056539) and Gladiolus hybrid Zefir leaves (a voucher specimen CWU0056538), as well as Iris hungarica Waldst. et Kit. rhizomes (a voucher specimen CWU0056534). Near November 2020, stigmas, flowers, leaves, and corms of C. sativus were taken from a plantation (a voucher specimen CWU0056541). The Herbarium validated and stored all voucher specimens. Fresh plant material was dried and pulverised in the open air.

- **Preparation of extracts**

Juno leaves, Gladiolus leaves, Crocus leaves, flowers, and corms, and Iris rhizome (100 g each) were extracted three times with distilled water (1 l) over a water bath for two hours each time. The extracts were filtered and chilled. The extracts were concentrated with Rotavapor, then dried thoroughly in a drying chamber and stored at 4°C until needed. Crocus stigma powder (5 g) was infused in a dark environment for 24 hours with hot distilled water (500 ml, 80°C). The extracted liquid was drained, and the remaining raw material was replaced with 500 mL distilled water and macerated for another 24 hours at 4°C. Maceration was carried out once again under the identical circumstances. The extracted materials were mixed, filtered, and dried at 80°C in a rotary evaporator. The powders were kept at 4°C in sealed containers for future use.

- **MTT assay**

The MTT test (EZ4U, Biomedica, Austria) was used to determine the antineoplastic activity of plant extracts on several tissue origin cell lines. The cells were plated overnight in 96-well plates with a volume of 100 µl at densities of 5,000 cells per well (substrate-dependent cells) or 10,000–15,000 cells per well (suspension cells). The cells were then cultured for 72 hours with aliquots of 100 µl of experimental extracts (0, 1, 10, and 100 µg/ml) and the reference drug doxorubicin (0, 1, and 10 µg/ml) in culture media. In addition, the MTT reagent (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide) was added to the cells according to the manufacturer's instructions. An Absorbance Reader Bio Tek ELx800 (BioTek Instruments, Inc., Winooski, VT) was used to measure the reaction's outcomes at 490 and 630 nm. The ratio of optical densities recorded for the wells with tested substances to the wells with control (non-treated) cells was used to

calculate the relative number of live cells (parts per unit). The extract concentration that reduced cell viability to 50% was estimated as the half-maximal inhibitory concentration (IC50) of the studied extracts. As a positive control, doxorubicin (Actavis, Romania) was chosen as a reference medicine. The extracts were applied to the cultivated cells at the stated concentrations (0–100 µg/ml) and left for 72 hours to work. GraphPad Prism 6 was used to analyse and visualise the data (GraphPad Software., San Jose, CA). The results are reported as the average standard deviation (SD) of three replications performed in two parallels (n = 6).

- **Culture of cells**

The Institute of Cancer Research at Vienna Medical University provided a collection sample of HL-60 human myeloid leukaemia cells (acute promyelocytic leukaemia) (Vienna, Austria). The Institute of Molecular Biology and Genetics, provided HepG2 human hepatocarcinoma cells (liver hepatocellular carcinoma), HCT116 human colon carcinoma cells (human colorectal carcinoma), and a sample of HaCaT human cell (normal human epidermal keratinocytes). The R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology provided collection samples of HeLa human cervix cancer cells (cervical adenocarcinoma) and MCF-7 human breast adenocarcinoma cells (Michigan Cancer Foundation-7). All cells were cultured in RPMI-1640 or Dulbecco's Modified Eagle's Medium with 10% foetal bovine serum supplementation (all from BioWest, France). The cells were grown at 37°C in a 5 percent CO2 environment.

IV. RESULTS

The anticancer activity of Iridaceae dry extracts was tested in vitro using the MTT assay against the following cell lines: The MTT test was used to investigate MCF-7 human breast adenocarcinoma cells, HCT116 human colon adenocarcinoma cells, HeLa cervical adenocarcinoma cells, HepG2 hepatocellular carcinoma cells, HL-60 acute promyelocytic leukaemia cells, and HaCaT normal human epidermal keratinocytes cells. Table 1 shows the findings of the analysis, which were represented as IC50. Iridaceae extracts contain a variety of antiproliferative properties. The activity of such extracts was decreased when compared to the reference drug doxorubicin; yet, several extracts exhibited a micromolar IC50 value.

The results revealed that the HL-60 line of human acute promyelocytic leukaemia cells were the most susceptible to the effect of all the plant extracts tested. With an IC50 value of 0.5 µg/ml, the dry Gladiolus leaf extracts (Table 1 and Fig. 1) demonstrated the maximum toxicity toward leukaemia cells. The anticancer activity of Juno leaf extracts and Iris rhizome extract was similar, with IC50 values of 3.5 and 3.6 µg/ml, respectively. Crocus corms extract has an IC50 value of 7.4 µg/ml. Even at 100 µg/ml, extracts of Crocus stigma, flowers, and leaves failed to reach the IC50 value. Crocin from Crocus stigma has previously been examined for its antitumor action on HL-60 human leukaemia cells, but only for the entire extract and not for myeloid leukaemia cell lines. As a result, the potential anticancer activity of Iridaceae extracts on HL-60 leukaemia cells may be categorised as follows: Iris rhizome = Gladiolus leaves = Juno leaves = Crocus corms

Plant extracts were also active in human colon cancer cells of the HCT116 line. Gladiolus leaf extract had an IC50 of 9.4 µg/ml, Iris rhizome had an IC50 of 42.3 µg/ml, Crocus corms had an IC50 of 74.6 µg/ml, and Crocus stigma had an IC50 of 94.8 µg/ml. The impact of saffron extract (0.2–1 mg/ml) in HCT116 colorectal cancer cells has been documented, although this study only comprises Crocus stigma extract. The IC50 values for stigma extract varied from 0.58 to 0.98 mg/ml, according to the study's findings. It's worth noting that the extracts' activity is directly influenced by the makeup of the active components in the raw material. Furthermore, the component composition may differ depending on where the plant is grown. This is likely why the activity of the saffron stigma extract is somewhat higher than that of the extract from Kashmir. Even at 100 µg/ml, extracts of Juno leaves and Crocus flowers and leaves failed to reach the IC50 threshold (Table 1 and Fig. 1). Gladiolus leaf extract was likewise the most active sample in this situation.

Table 1: Antiproliferative activity of tested extracts, IC_{50}, µg/ml.

No	Dry extract	MCF-7	HCT116	HeLa	HepG2	HL-60	HaCaT
1	Gladiolus leaves	>100	9.4	>100	>100	0.50	>100
2	Juno leaves	>100	>100	>100	>100	3.5	>100
3	Iris rhizome	>100	42.3	78.7	>100	3.6	>100
4	Crocus stigma	>100	94.8	>100	>100	>100	>100
5	Crocus corms	>100	74.6	>100	>100	7.4	>100
6	Crocus flowers	>100	>100	>100	>100	>100	>100
7	Crocus leaves	>100	>100	>100	>100	>100	>100
	Dox	0.51	0.79	0.21	0.34	0.11	0.7

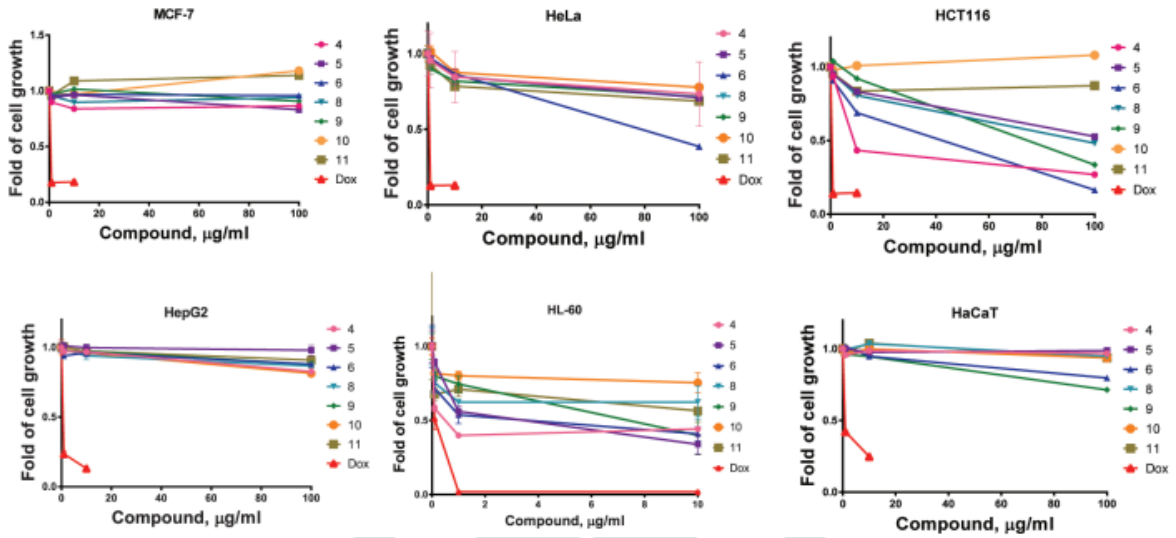

Figure 1. Cytotoxicity of all tested dry extracts toward different cell lines. After a total experimental time (72 hours), cell vitality was detected by the MTT assay. 1— Gladiolus leaf extract; 2—Juno leaf extract; 3—Iris rhizome extract; 4—Crocus stigma extract; 5—Crocus corm extract; 6—Crocus flower extract; 7—Crocus leaf extract.

With an IC50 of 78.7 µg/ml, iris rhizome extract was found to be hazardous to human cervical cancer of the HeLa line. HeLa cells were not harmed by any of the other plant extracts tested (Table 1 and Fig. 1). The extracts of the examined plants revealed the least sensitivity in MCF-7 human breast adenocarcinoma cells and HepG2 human hepatocellular carcinoma cells. The extracts tested did not exhibit an IC50 of 100 µg/ml. When compared to the action of plant extracts, doxorubicin had a greater cytotoxic effect on all cell lines examined.

The extracts of the plants tested were not harmful to normal human epidermal keratinocytes of the HaCaT line. Crocus corms extract inhibited the development of HaCaT cells to a maximum of 28.6%, whereas Juno leaf extract inhibited it to a minimum of 2%. (Table 1 and Fig. 1). The doxorubicin IC50 value for HaCaT cells was 0.7 µg/ml. The cytotoxic activity of Gladiolus leaf and Iris rhizome extracts on HL60 human acute promyelocytic leukaemia cells and HCT116 colon adenocarcinoma cells was therefore the highest. The concentration-dependent activity of C. sativus flower acetone extract (50–200µ g/ml) on HaCaT cells had previously been reported. The acetone floral extract, on the other hand, has a more hazardous solvent than the

aqueous extract. In subsequent research, we will need to raise the dose of Iridaceae plant extract and repeat the experiment, as other writers use a pre-high concentration of plant extract as well.

Several representatives of the Iridaceae genus of plants have been examined for anticancer and cytotoxic properties in this study. The cytotoxicity of Gladiolus quartinianus (40 g/ml) methanol extract against CCRF-CEM leukaemia cells, which had the lowest IC50 values below 30 µg/ml. In addition, G. quartinianus extract was reported to induce apoptosis in CCRFCEM cells when the mitochondrial membrane potential was lost. With IC50 of 128 and 237 mg/ml, respectively, the methanol extract of Iris kashmiriana rhizome (400 mg/ml) demonstrated a significant cytotoxic impact against A549 human epithelial cancer cell lines and Caco-2 epithelial cell lines (Asif et al., 2013). On human colon adenocarcinoma cells, ethanol extracts from C. sativus corms, flowers, and leaves had an antiproliferative effect (Caco2). According to their findings, the flower and leaf extract (ED5 0.42 mg/ml) significantly reduced Caco-2 cell activity. In additional studies, the stigma aqueous extract of C. sativus prevented cancer development in dosages of 100, 150, and 175 mg/kg. The cytotoxic activity of a glycoconjugate derived from saffron corms was previously shown in connection to the HepG-2 hepatocellular carcinoma cell line and the Hep-2 laryngeal carcinoma cell line. Crocin was discovered to be cytotoxic in HeLa and MCF-7 cell lines in another investigation. However, the studies provided here are mostly focused on the specific chemicals found in C. sativus and its stigma.

On MCF-7 human breast cancer cell line, HepG2 human hepatocellular carcinoma cell line, HCT116 human colon adenocarcinoma cell line, HeLa cervical adenocarcinoma cell line, HL-60 acute promyelocytic leukaemia cells line, and HaCaT normal human epidermal keratinocyte cell line, the anticancer activity of J. bucharica leaves, G. hybrid Zefir leaves, I. As a result, the findings suggest that Iridaceae plants have the potential to cure cancer, although further research is needed.

V. CONCLUSION

Plants have traditionally played a vital role in the treatment and prevention of cancer and other illnesses in folk and clinical medicine in various nations. Furthermore, natural components from plants are utilised in the development of novel medications at the level of synthetic molecules. Plants of the Iridaceae family (J. bucharica, G. hybrid Zefir, I. hungarica, and C. sativus) are herbaceous plants that have been used in European folk medicine for antitumor, anti-inflammatory, anticonvulsant, analgesic, antioxidative activity, antipyretic, and other purposes. In the MTT experiment, gladiolus leaf extract has notably high anticancer activities against human leukaemia and colon adenocarcinoma cells. On human cervical cancer cells, iris rhizome dry extracts had a reduced activity. The HaCaT line of normal human epidermal keratinocytes showed little toxicity to several plant extracts. The research into the action mechanism of the extracts under investigation will continue. In vitro investigations have indicated that these herbal extracts have potential anticancer action, which can be exploited to develop new drugs for the prevention and treatment of leukaemia, colon cancer, and cervical malignancies. This is the first study to look at the anticancer properties of plant extracts from the Iridaceae family.

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