https://doi.org/10.33472/AFJBS.6.13.2024.1448-1456

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Potential Anti-İnflammatory and Apoptotic Effect of Carthamus Lanatus Extract in Human Leukemia Cell Line THP-1

Melakhessou Mohamed Akram[1*], Marref Salah Eddine[1], Becheker Imene[2]

[1]Laboratory of Biotechnology of Bioactive Molecules and Cellular Physiopathology (LBMBPC), Faculty of Natural and Life Sciences, University Batna 2, Fesdis, Batna, 05078, Batna, Algeria

[2]Department of Nature and Life Sciences, Laboratory of Research in Biodiversity Interaction Ecosystem and Biotechnology (LRIBEB), University 20 August 1955, Skikda, 21000, Algeria

Corresponding Author: Melakhessou Mohamed Akram
Laboratory of Biotechnology of Bioactive Molecules and Cellular Physiopathology (LBMBPC), Faculty of Natural and Life Sciences, University Batna 2, Fesdis, Batna, 05078, Batna, Algeria
Email : m.melakhessou@univ-batna2.dz

Author contributions: Concept – B.Y., M.M.A.; Design – B.Y., M.M.A.; Analysis and/or Interpretation – B.Y., M.M.A.; Literature Search – B.Y., M.S.E; Review – B.Y., B.I.

Article Info

Volume 6, Issue 13, July 2024

Received: 02 June 2024

Accepted: 30 June 2024

Published: 24 July 2024

doi: 10.33472/AFJBS.6.13.2024.0000-0000

ABSTRACT:

Cancer is a leading cause of death and a healthcare challenge in the world. Hence, this work was conducted to determine the *in vitro* anticancer property and also the molecular mechanism of hydro-alcoholic extract of *Carthamus lanatus* L (HACL) whole plant in a human cancer cell line, THP-1 (human leukemia cell line). *In vitro* cytotoxic potential of the extract was examined using the standard MTT (3-(4,5-dimethylthiazole)-2,5-diphenyltetrazolium bromide) method and water-soluble tetrazolium salt (*WST-1*) in human leukemia cell culture. In turn, the gene expression levels of B-cell lymphoma 2 (*Bcl-2*), tumor necrosis factor-alpha (*TNFα*), interleukin 6 *(IL-6*), cysteine-dependent aspartate-specific peptidase (Caspase 3 and 8) and programmed cell death 4 were assessed by applying RT-qPCR. The MTT and WST-1 assays data showed that the hydro-alcoholic extract of *C. lanatus* had an antiproliferative effect on human leukemia with IC_{50} of 523.5±2.23 and 497.23±3.15 µg/mL, respectively. The gene expression levels of tumor necrosis TNFα, IL-6, Bcl-2, PDCD4, Caspase 3, and caspase 8 were assessed by applying RT-qPCR. Our data showed that HCAL induces anticancer effect by down-regulating the anti-apoptotic (Bcl-2) and pro-inflammatory (IL-6 and TNFα) genes expression. On the contrary, pro-apoptotic PDCD4, caspase 3 and caspase 8 genes were significantly up-regulated in THP-1 cells. However, based on the up-regulation of caspases, two distinct types of apoptotic pathways are proposed. The extrinsic pathways or ROS production involvement, however, cannot be excluded. It would be necessary to do more research to demonstrate these constituents using a bioguided fractionation. This will enable the isolation, identification of the active principles responsible of other regulators/signals involved in the cytotoxic and apoptotic effect of the *C. lanatus* extract, and the elucidation of their mechanism of action.

Keywords: *Carthamus lanatus L,* in human Leukemia Cells, pro-apoptotic, anti-apoptotic.

1. INTRODUCTION

Leukemia is a blood-related cancer defined by widespread bone marrow invasion and altered hematopoietic progenitors. Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML) are the four main forms of leukemia. Leukemia contributed roughly 2.5% and 3.1%, respectively, to all new cancer cases and deaths worldwide in 2020 [1]. There are differences in the risk of leukemia between groups based on age, gender, and geography [2]. These discrepancies may be related to variations in the frequency of certain genetic and environmental risk factors for leukemia.

Many chemotherapeutic drugs, surgical techniques, and chemotherapy methods are currently used in the treatment of cancer. On the other hand, using chemotherapy medications has adverse effects. Consequently, there has been a lot of interest in creating novel anti-cancer substances derived from traditional medicine and medicinal plants, especially because of their demonstrated ability to enhance the therapeutic process [3]. The use of traditional medicinal herbs has long been shown to be beneficial in the treatment of a number of cancer disorders, and it is currently one of the most fundamental ways to treat cancer [4].

Bioactive substances such quercitin, kaempferol, isoquercitrin, and luteolin have a therapeutically substantial antitumor impact and can be applied to a variety of cancer disorders [5]. For millennia, a wide range of medicinal plants from many plant families have been employed in medicine, nutrition, and cosmetics. *Carthamus lanatus* L, growing in the Mediterranean region, is a biennial plant [6]. Previous research on this plant has demonstrated its

significance because it contains a variety of components with different chemical properties. Studies on phytochemistry revealed the existence of flavonoids, lipids, aromatic acids, sterols, triterpenes, and volatiles as the major secondary metabolites [7]. Alkaloids, tannins, and saponins have all been documented recently the most significant biological activity for C. *lanatus*. has been documented, and they include anticancer, analgesic, anti-inflammatory, antioxidant, and sedative properties [8].

The purpose of this study was to assess the effects of C. *lanatus* on the cell viability of THP-1 cancer cell lines. The gene expression levels of pro-apoptotic (PDCD4, caspase 8, caspase 3), pro-inflammatory (IL-6, TNFα), and anti-apoptotic (BCL2) genes involved in apoptosis were analyzed.

2. MATERIALS AND METHODS

Collection of plant and extraction procedure

The collection of C. *lanatus* whole plant was done in Batna District in eastern Algeria in June 2019 (flowering season, at Arris, Batna, 35°15’23’’N; 6°20’13’’E). Taxonomic identification was achieved at the Agronomic Institute, University of Banta, Algeria (by botanist Pr. Bachir Oudjehih). The whole plant was ground after being dried naturally (under shade for 2 weeks). The plant was powdered by using a laboratory mill.

Extraction techniques

To obtain the Hydro-alcoholic extract, a maceration technique was applied. The powdered whole plant was stirred in a methanol/water 80:20 (v/v) mix (in a magnetic shaker for 24h at ambient temperature). The obtained hydro-alcoholic extract was then dried by using a vacuum evaporator and lyophilizing was performed to obtain Hydro-alcoholic extract using a freeze dryer.

Cell culture

The human monocytic THP-1 (ATCC®, TIB-202TM, Manassas, VA, USA) non-adherent monocyte cell line was grown in RPMI 1640 medium supplemented with 2 mM of l-glutamine, 10% of heat-inactivated foetal calf serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 0.25 µg/mL of amphotericin B and incubated in a humidified mixture of air (95%) and CO2 (5%) at 37°C and split every 4 days [9].

Gene expression analysis by real-time PCR (qRT-PCR)

The expression of the following genes by THP-1 cells was assessed: nuclear factor kappa-light-chain enhancer, the gene expression of

In undertaking this task, total RNA was extracted from 1.5×10^6 THP-1 cells unexposed or exposed for 24 h to 500 µg/mL of *HACL by* TRIzol® Reagent (Invitrogen, La Jolla, CA). The degradation and purity of isolated RNA were determined ined by measuring optical densities using Bio- Specnano (Shimadzu Corporation, Kyoto, Japan) and capillary electrophoresis using RNA 6000 Nano® kit and the Bioanalyzer TM 2100. Moreover, iScript™ complementary Synthesis Kit (Bio-Rad, France) was employed for reverse transcription. In addition, cDNA synthesis was performed with 100 ng total RNA. Gene expressions were explored by RT-qPCR via iQTM SYBR Green® Supermix in a Stratagene Mx3000p system (Agilent Technologies). Herein, 4 µL of each cDNA sample was amplified in a PCR reaction (final volume of 20 µL) containing 10 µL of PCR reagent and 300 nM of each gene specific primers (Table 1). For all samples the following conditions were used: an initial heat-denaturing step at 95°C for 5 min followed by 40 cycles of 95°C for 15 s, annealing at 60°C

for 40 s, elongation and signal acquisition at 72°C for 40 s. To approve the amplification of specific transcripts, melting curve profiles were produced at the end of each reaction, and if two or more peaks were present, the results were excluded. Water was used as a negative control for each PCR run. For each gene, amplifications were performed from three prepared samples. Gene expression levels were normalized by comparison to ribosomal protein (RPL13) housekeeping genes. Fold change (FC) of gene expression was calculated by applying the $2^{-\Delta\Delta Ct}$ method [10].

Table 1. Gene expression changes in response to HACL exposure of THP-1 human leukemia cells. Cells were exposed to 500 µg/mL of H for HACL 4 h

Functional class	Gene	Sequence
Apoptosis	ACTB (internal control)	F :5'-TTGGCAATGAGCGGTTCC-3' R :5'-GTACTTGCGCTCAGGAGGAG-3
	PDCD4	F : 5'-'AGACCAAATGAAAAGAGGTTATGAG-3' R : 5'-GCCCCTTGAAGGACAAAGAT-3'
	BCL2	F : 5'-GAGGATTGTGGCCTTCTTTG-3' R : 5'-GCATCCCAGCCTCCGTTAT-3'
	CASP8	F : 5'-GAAAGGGTGGAGCGGATTAT-3' R : 5'-GCTTCCTTTGCGGAATGTAG-3'
	CASP3	F : 5'-TATGGTTTTGTGATGTTTGTCC-3' R : 5'-TAGATCCAGGGGCATTGTAG-3'
Inflammation	IL-6	F:5'-CAATTCTGGTATTCTTTCCCA-3' R : 5'-GCAAGTCTCCTCATTGAATCC-3'
	TNF-α	F:5'-TAGCCCATGTTGTAGCAAACC-3' R : 5'-GATGGCAGAGAGGAGGTTGA-3'

Data analysis

GraphPad Prism version 8.3.0 (GraphPad Software) was used to perform statistical analysis on the acquired data, and a 2-sided P <.05 was deemed statistically significant. The obtained data were analyzed using the one-way ANOVA approach, and when necessary, the Pearson correlation coefficient was run.

3. RESULTS

THP-1 Cell Viability Assays

The vitality of THP-1 cells was reduced by HACL exposure for 24 hours in a dose-dependent manner using WST-1 test, reaching 60% of the control at 500 µg/mL. Simultaneously, the MTT test revealed a notable reduction in the quantity of THP-1 cells (down to 50% of control after exposure to 500 µg/mL) (Fig. 1).

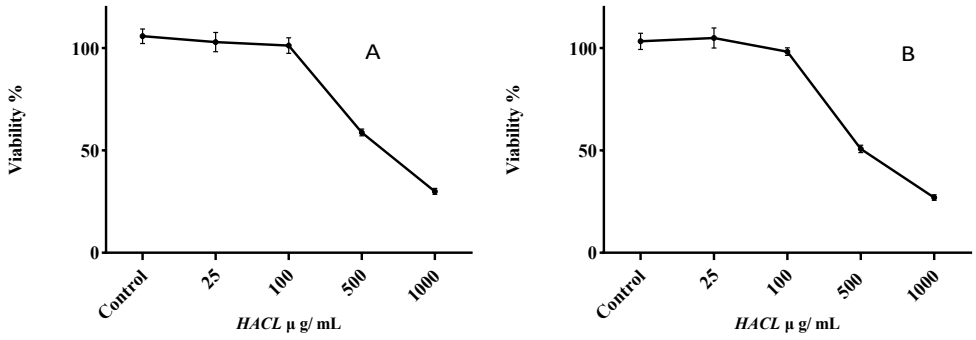

Figure 1. Cytotoxicity of *HACL on* THP-1 cells. A: The cytotoxicity was evaluated through MTT assay, B; The cytotoxicity was evaluated through WST-1 assay. Data represents the means ± SEM (N=4; n=6)

These results showed that an adequate concentration of HACL was needed to decrease cell growth. The results from the WST-1 and MTT also demonstrated that the decrease in the viability of THP-1 cell line was dose-dependent. The IC50 value corresponding to HACL is shown in Table 2.

Table 2. The IC_{50} Values (µg/mL) of *HACL* on THP-1 Cells.

Assay	**IC50**
MTT	523.5±2.23
WST-1	497.23±3.15

Gene Expression Assay RT-qPCR

The gene expression analyses via RT-qPCR revealed that after 24 h, the anti-apoptotic Bcl-2 gene was significantly down-regulated in human leukemia cells. On the contrary, pro-apoptotic PDCD4 and caspase 8 genes were significantly up-regulated compared to untreated control cells (Fig2. and Table 3). Results were represented as FC.

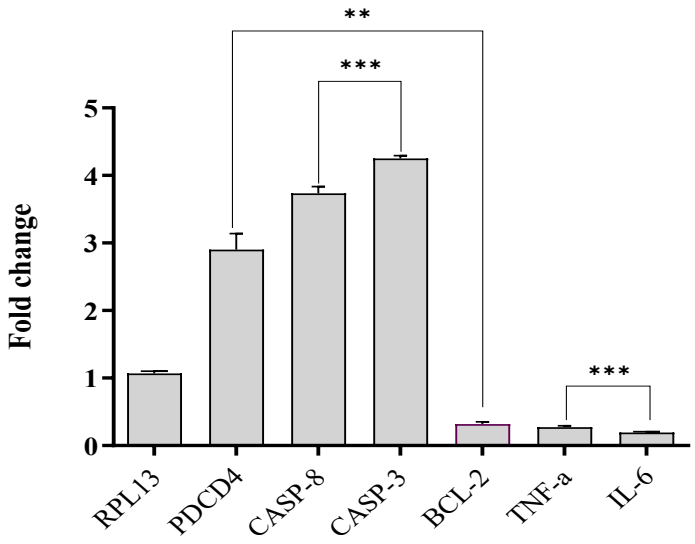

Figure 2. Gene expression levels of the selected genes as a percent of the control. For quantitation of RT-qPCR results: * p<0.05, ** p<0.01, *** p<0.001

Table 3. Gene expression changes in response to HACL exposure of THP-1 human leukemia cells. Cells were exposed to 500 µg/mL of H for HACL 4 h

Gene	**Fold change**	**P value**
PDCD4	2.90±0.23	<0.01
CASPASE 8	3.73±0.09	<0.001
CASPASE 3	4.25±0.03	<0.001
BCL-2	0.32±0.032	<0.01
IL-6	0.19±0.002	<0.001
TNF-a	0.27±0.01	<0.001

4. DİSCUSSİON

AML, or acute myeloid leukemia, is a very aggressive hematological cancer that is linked to the accumulation of molecular and cytogenetic abnormalities in progenitor and/or hematopoietic stem cells (HSPCs) [11]. Leukemic stem cells (LSCs) arise as a result of this illness. The natural way that cells die, called apoptosis, presents a viable target for anticancer treatment. Caspases are used by the extrinsic and intrinsic pathways to cleave hundreds of proteins, hence causing apoptosis. The apoptotic process is commonly blocked in cancer by a multitude of strategies, such as overexpression of antiapoptotic proteins and under expression of proapoptotic proteins. Chemotherapy, the most widely used anticancer treatment, is intrinsically resistant to many of these alterations. Therefore, plant-derived substances that activate the apoptotic pathway to demonstrate anticancer action are promising novel anticancer treatments [11]. To confirm the cytotoxic effect of HACL on monocytic leukemia cell line, viability assays were performed. In this study, MTT and WST-1 assays showed that the viability of THP-1 cells decreased with increasing doses of HACL.

Previous research has demonstrated the anticancer properties of the genus *Carthamus. Carthamus tinctorius* seed dramatically reduces the growth of human colon cancer cells (HCT116, SW480, LoVo, and HT-29). It inhibits proliferation by promoting proteasomal degradation of cyclin D1 via ERK1/2-dependent threonine-286 phosphorylation of cyclin D1 [12]. Furthermore, *Carthamus tinctorius* polysaccharide strongly suppressed the growth of the human breast cancer cell line (MCF-7). It also causes cell death by decreasing Bcl-2 expression while increasing Bcl-2-related protein X expression. Furthermore, in MCF-7 human breast cancer cells treated with *Carthamus tinctorius* polysaccharide, matrix metalloproteinase-9 expression was significantly reduced while tissue inhibitor of metalloproteinase-1 expression increased. As a result, it inhibits the spread of MCF-7 breast cancer cells [13]. Furthermore, the effect of this polysaccharide on tongue squamous cell cancer (TSCC) has also been studied. This polysaccharide may slow the progression of TSCC by modulating the expression of Bcl-2, COX-2, and Bax and inhibiting caspase-3 [14].

In order to investigate the apoptotic pathway of THP-1 cells, the gene expression levels of Bcl-2, caspase-8, caspase-3, PDCD4, IL-6, and TNF-α were analyzed. The Bcl-2 family is recognized for its significant function in controlling how cells react to a range of apoptotic signals. Bcl2-associated X protein (Bax) stimulates cell death, whereas Bcl-2 inhibits apoptotic cell death [15]. The Bcl-2 to Bax pre-ratio establishes a cell's vulnerability to a particular apoptotic trigger. Even though the Bcl-2/Bax ratio is innate to the cell, it seems that extrinsic factors can trigger apoptosis because they can reset the ratio by either upregulating bax or downregulating Bcl-2 [16]. Bcl-2 was shown to be downregulated in our data. These results imply that a reduction in Bcl-2 might play a significant role in the HACL-treated monocytic leukemia cell line THP-1cells' apoptotic pathway.

On the other hand, overexpression of PDCD4 activates the pro-apoptotic member of the Bcl-2 protein family Bax, which in turn triggers the release of cytochrome C from mitochondria and the activation of caspases 8, 9, and 3, thereby inducing apoptosis in hepatoma cells, though the exact mechanism of Bax activation remains unclear [12]. Apoptosis is also reportedly induced by PDCD4 knockdown. According to Eto et al., the deletion of PDCD4 upregulated pro-caspase 3 expression, which in turn caused an increase in active caspase 3 levels and the triggering of apoptosis in HeLa cells in the absence of apoptotic triggers[14]. In this study, we observed an over expression level of PDCD4. These results suggest that an increased level of PDCD4 may induce apoptosis.

It has also been reported that Caspases, or cysteine aspartyl-specific proteases, are a class of cysteine proteins that break target proteins to cause apoptosis [17]. Because caspase proteases can

cleave hundreds of different proteins, they are necessary for apoptosis to occur successfully. Three executioner caspases (caspase-3, 6, and 7) and four initiator caspases (caspase 2,8, 9 and 10) exist [16]. The target proteins are cleaved by the executioner caspases, ultimately resulting in cell death. Because the pathways are so tightly controlled, apoptosis can only happen in response to signals. The Bcl-2 protein family, which consists of proapoptotic effector proteins, proapoptotic BH3-only proteins, and antiapoptotic Bcl-2 proteins, specifically regulates the intrinsic route [18]. In our experiment, we observed an over-expression of caspase 3 and caspase 8. The expression of caspase 3 and caspase 8 might be an important pathway of cell apoptosis.
Pro-inflammatory cytokine IL-6 is reported to partially enhance AML cell proliferation and to be raised in patient plasma, according to published literature [17]. Our study data revealed that there is a hard down-regulation of the gene expression level l of IL-6 in the treated THP-1 cells. As previously documented, TNF α is a pro-inflammatory cytokine that promotes AML cell chemoresistance and LSC proliferation *in vitro*. Moreover, it was discovered that AML patients have higher levels of PB [19,20,21] . In the THP-1 cell treatment sets, our data show a decrease in gene expression of TNF α. It is revealed that alkane-6,8-diols, which are volatile components from the seed of CL, can prevent tumor promotion in two-stage mouse skin carcinogenesis caused by 12-O-tetradecanoylphorbol-13-acetate. Furthermore, the formation of melanin by *Streptomyces bikiniensis* and B16 melanoma cells is significantly inhibited by N-feruloyl and N-(p-coumaroyl) serotonin [22,23]. Potential anticancer effects of these substances have been suggested. For cerebral ischemia injury, CL is additionally neuroprotective both *in vivo* and *in vitro* [24]. N-(p-coumaroyl) serotonin and N-(p-coumaroyl) tryptamine, the active components in CL, have been demonstrated to potently suppress the generation of proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8, and TNF-α) in human monocytes stimulated with lipopolysaccharide [25].
With these initial cell culture results, the next step would be to prospectively test the plant extract in murine models whose strain allows leukaemia to be recreated and to confirm whether the effect is consistent or whether it would represent a step forward in a likely treatment that complements chemotherapy without interfering with it.

5. CONCLUSİON

The findings of this study showed that C. lanatus extract significantly inhibits cancer growth and induces apoptosis in THP-1 cells. The modulation of the examined genes and molecular signaling pathways provided evidence of the phytochemicals of the C. lanatus extract's anti-cancer action. This finding provides a path forward for more research to validate and elucidate the possible function of *C. lanatus* extract in cancer medication development. Future studies should investigate these mechanisms in animal models.

6. REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer J Clin 2020;71(3):209–49. DOI: 10.3322/caac.21660.
2. Lim JY, Bhatia S, Robison LL, Yang JJ. Genomics of Racial and Ethnic Disparities in Childhood Acute Lymphoblastic Leukemia. Cancer 2014;120(7):955–62. DOI: 10.1002/cncr.28531.

3. Greenwell M, Rahman PK.2015. Medicinal Plants: Their Use in Anticancer Treatment. Int J Pharm Sci Res.2015; 6(10):4103-12. https://doi.org/10.13040/ijpsr.0975-8232.6(10).4103-12.
4. Ali-Shtayeh MS, Jamous RM, Salameh NM, Jamous RM, Hamadeh AM.2016. Complementary and alternative medicine use among cancer patients in Palestine with special reference to safety-related concerns. J Ethnopharmacol. 2016;187:104-22. https://doi.org/10.1016/j.jep.2016.04.038.
5. Abu ElKhair RM, Omran GAE, YasserM , & Seif ElDin AA.Qualitative GC-MS analysis and antimicrobial activity of volatiles from Carthamus lanatus (L.) growing in Egypt. Records of Pharmaceutical and Biomedical Sciences,2020; 4(1), 6-12.DOI: 10.21608/rpbs.2019.15957.1036.
6. Mohammadpanah M, Mojodi E, Ehsani R. The Synthesis and Characterization of Liposomal Nano-Carriers Loading Lavandula angustifolia Essential Oil to Affect Breast Cancerous Cell-Lines. Yafteh. 2020;22(1):84-95. Persian. https://sid.ir/paper/79942/en.
7. Pate KK & Narasimhacharya AVRL. Potentiality of phytochemical compounds isolated and identified from Jatropha curcas L. against Odontotermes obesus Rambur. Journal of Natural Pesticide Research, 2022;2, 100009. https://doi.org/10.1016/j.napere.2022.100009.
8. Lindenboim L, Zohar H, Gundersen GG, Worman HJ, & Stein R. LINC complex protein nesprin-2 has pro-apoptotic activity via Bcl-2 family proteins. Cell Death Discovery, 2024;10(1), 29. https://doi.org/10.1038/s41420-023-01763-w.
9. Ashour HA, Esmail SEA, & El-Attar AB. . Effect of different nitrogen forms and bio-treatments on the growth and seed yield of downy safflower (Carthamus lanatus). Advances in Horticultural Science, 2023;37(2), 185-196. DOI: 10.36253/ahsc-12938.
10. Akram MM, Naima B, Eddine MS, Joubert O, Doumandji ZM, & Safar R. Gene expression levels of selected factors in monocytic leukemia cell line THP-1 upon treatment with n-butanol extract of atractylis flava desf against cancer. Journal of Young Pharmacists, 2019;11(1), 36. DOI: 10.5530/jyp.2019.11.8
11. Eddine MS, Naima B, Akram MM, Joubert O, Doumandji ZM, & Safar R. Anti-cancer Activity and Gene Expression Responses to Methanol Extract of Gladiolus segetum in THP-1 Human Monocytic Leukemia Cells. Journal of Young Pharmacists, 2019;11(1), 51. DOI: 10.5530/jyp.2019.11.11.
12. Park, GH, Hong SC, Jeong JB. Anticancer Activity of the Safflower Seeds (Carthamus tinctorius L.) through Inducing Cyclin D1 Proteasomal Degradation in Human Colorectal Cancer Cells. Korean J Plant Res 2016; 29: 297-304.
13. Luo Z, Zeng H, Ye Y, Liu L, Li S, Zhang J, Luo R. Safflower polysaccharide inhibits the proliferation and metastasis of MCF-7 breast cancer cell. Mol Med Rep 2015; 11: 4611-6.
14. Zhou H, Yang J, Zhang C, Zhang Y, Wang R, Li X, Zhang S. Safflower polysaccharide inhibits the development of tongue squa- mous cell carcinoma. World J Surg Oncol 2018; 16: 167.
15. Liphaus BL, Silva SC, Palmeira P, Silva CA, Goldenstein-Schainberg C, & Carneiro-Sampaio M. Reduced expressions of apoptosis-related proteins TRAIL, Bcl-2, and TNFR1 in NK cells of juvenile-onset systemic lupus erythematosus patients: relations with disease activity, nephritis, and neuropsychiatric involvement. Frontiers in Immunology, 2024;15, 1327255. https://doi.org/10.3389/fimmu.2024.1327255.
16. Huang J, Chan SC, Ngai CH, Lok V, Zhang L, Lucero-Prisno DE 3rd, Xu W, Zheng ZJ, Elcarte E, Withers M, Wong MCS.2022. Disease Burden, Risk Factors, and Trends of

Leukaemia: A Global Analysis. Front Oncol. 2022;22; 12:904292. doi: 10.3389/fonc.2022.904292.

17. Ekundayo BE , Obafemi TO, Adewale OB, Obafemi BA, Oyinloye BE, & Ekundayo SK. Oxidative Stress, Endoplasmic Reticulum Stress and Apoptosis in the Pathology of Alzheimer's Disease. Cell Biochemistry and Biophysics, 2024;1-21. https://doi.org/10.1007/s12013-024-01248-2.
18. Chesnokov MS, Mamedova AR, Zhivotovsky B, & Kopeina GS. A matter of new life and cell death: programmed cell death in the mammalian ovary. Journal of Biomedical Science, 2024;31(1), 1-34. https://doi.org/10.1186/s12929-024-01017-6
19. Konopleva M, Cardama A, Kantarjian H, Cortes J. Molecular biology and cytogenetics of chronic myeloid leukemia. Neoplastic Diseases of the Blood. 2018;29-47. https://doi. org/10.1007/978-3-319-64263-5_4.
20. Islam M, Mohamed EH, Esa E, Kamaluddin NR, Zain SM, Yusoff YM, et al. Circulating cytokines and small molecules follow distinct expression patterns in acute myeloid leukaemia. Br J Cancer. 2017;117(10):1551-6. https://doi. org/10.1038/bjc.2017.316.
21. Karimdadi Sariani O, Eghbalpour S, Kazemi E, Rafiei Buzhani K, Zaker F. Pathogenic and therapeutic roles of cytokines in acute myeloid leukemia. Cytokine. 2021;142:155508. https://doi.org/10.1016/j.cyto.2021.155508.
22. Savage SA, Walsh MF.2018. Myelodysplastic syndrome, acute myeloid leukemia, and cancer surveillance in fanconi anemia. Hematol Oncol Clin North Am. 2018;32(4):657-68. https://doi.org/10.1016/j.hoc.2018.04.002.
23. Nazir M, Arif S, Ahmed I, & Khalid N.Ç Safflower (Carthamus tinctorius) seed. Oilseeds: Health Attributes and Food Applications, 2021;427-453. https://doi.org/10.1007/978-981-15-4194-0_17.
24. Federica Z, Luciana G, Angelini SB, Lara F, Clarissa C, Federico F, Angela V, Alessandro R, Andrea M and Silvia Tavarini.2022. Industrial Crops and Products, 2022; 184- 115042 https://doi: 10.1016/j.indcrop.2022.115042.
25. Zaiachuk M, Suryavanshi SV, Pryimak N , Kovalchuk I, & Kovalchuk O. The Anti-Inflammatory Effects of Cannabis sativa Extracts on LPS-Induced Cytokines Release in Human Macrophages. Molecules, 2023;28(13), 4991. https://doi.org/10.3390/molecules28134991.
26. Zhou J, Sun F, Zhang W, Feng Z, Yang Y, & Mei ZNovel insight into the therapeutical potential of flavonoids from traditional Chinese medicine against cerebral ischemia/reperfusion injury. Frontiers in Pharmacology, 2024;15, 1352760. https://doi.org/10.3389/fphar.2024.1352760