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Original Article

Apoptotic Effects of *Hypericum dogonbadanicum* on Lung and Gastric Cancer Cell Lines

Seyyedeh Mahdokht Maddah, Golaleh Mostafavi*, Masoud Vaali, Ehsan Ranjbar

Department of Biology, YI.C., Islamic Azad University, Tehran, IRAN.

ABSTRACT

Aim/Background: Cancer is the second deadliest disease worldwide. Medicinal plants have been recognized for their cytotoxic and antitumor properties. This study aimed to assess the potential anticancer effects of the hydroalcoholic extract and essential oil of an Iranian endemic species, *Hypericum dogonbadanicum,* on AGS, A549 and a normal cell and the novel investigation of changes in *caspase-3* and *caspase-8* genes expression level. **Materials and Methods:** The essential oil and extract were extracted using Clevenger and Maceration techniques respectively. Their components were determined employing GC/MS and HPLC methods. The evaluation of gene expression levels was accomplished by a quantitative PCR method. **Results:** According to MTT assay data, it was observed that the IC_{50} value for the essential oil was 200 µg/mL. However, for the extract, the IC_{50} values were different, with a value of 50 µg/mL after 72 hr of treatment in A549 cells and a value of 200 µg/mL after 48 hr of treatment in AGS cells. The Apoptosis analysis showed a notable rise in the numbers of both early and late apoptotic cells in both cell lines upon treatment with the extract and essential oil. Furthermore, when exposed to the extract, there was an observed overexpression pattern in both *caspase-3* and *caspase-8* genes in both cell lines. However, the essential oil effects led to significant upregulation of these genes only in AGS cells. **Conclusion:** Based on our findings, *H. dogonbadanicum* extract and essential oil give promising results as apoptotic inducers for both cancer cell lines via targeting and increasing *caspase-3* and *caspase-8* gene expression.

Keywords: Cytotoxic, Essential oil, Ethanolic extract, Gene expression, *Hypericum dogonbadanicum*.

Correspondence:
Golaleh Mostafavi
Department of Biology, YI.C., Islamic Azad University, Tehran, IRAN.
Email: golaleh.m@gmail.com; golaleh.mostafavi@iau.ac.ir

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INTRODUCTION

Cancer, which is the second leading cause of death worldwide, presents a significant challenge to improving global life expectancy.[1] It is often regarded as the primary or secondary leading cause of death for individuals under the age of 70.[2] In 2020, lung cancer was recognized as the second most prevalent form of cancer and the primary cause of cancer-related deaths worldwide. It accounted for over 2.2 million newly diagnosed cases and resulted in approximately 1.9 million deaths.[3] The majority of lung cancers can be classified into two major histological groups: Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). These two categories help differentiate lung cancers based on their distinct characteristics and treatment approaches.[4] Gastric cancer is recognized as the fifth most commonly diagnosed cancer and the fourth leading cause of cancer-related death globally.[5] Even with recent advancements in the early detection and treatment of cancer, the majority of patients are diagnosed at an advanced stage and suffer a significantly poorer prognosis than those in the early stages.[6-8] *Caspases* are indeed proteolytic enzymes that is important in regulating cell death and inflammation. They are known to have well-defined functions in apoptosis (Programmed cell death). *Caspases* can act as initiators or executioners in the apoptotic process.[9] The initiator *caspase-8* is involved in the activation of the cascade responsible for receptor-induced cell death. Its active form can stimulate the activity of executioner *caspase-3* through the extrinsic death pathway.[9,10] Indeed, *caspases*, have significant roles in orchestrating apoptosis, regulating cell death pathways and mediating both extrinsic and intrinsic apoptotic pathways. Their activation and involvement in these pathways are critical for the execution of programmed cell death. Most common cancer treatments induce apoptosis indirectly to eliminate the cancer cells by applying such *caspases*.[11-13] Due to employing various chemical medicines for cancer treatment that their nonselective toxic properties can destroy a high percentage of normal cells, applying plant compounds as natural resources could be an appropriate successor.[14-17] The various species of *Hypericum* have been traditionally used for the treatment.[18] The

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EOs of different *Hypericum* species such as *H. perforatum, H. perfoliatum and H. scabrum* have been investigated during the last decade. The *Hypericum* EOs comprise major phytocomponents including α-pinene, β-pinene, E-caryophyllene, germacrene D, spathulenol and caryophyllene.[19] Moreover, in *Hypericum* extracts, approximately 150 different components have been discovered, with flavonol glycosides being the predominant group of compounds in *Hypericum perforatum.*[20] Hypericin and pseudohypericin belonging to the naphthodianthrones group, are found in the crude extract of flowers and leaves. Additionally, adhyperforin and hyperforin, classified as phloroglucinols, are exclusively present in the pistils and green fruit.[21] *Hypericum dogonbadanicuin* Assadi is a medicinal shrub that is endemic to the mountains of Dogonbadan in southwest Iran (Kuhkiluye and Boyer-Ahmad province).[22] Earlier research has indicated that it has the potential to act as an anti-depressant, sedative, nerve tonic and antioxidant.[23,24] Other research has also focused on studying the cytotoxic properties of various *Hypericum* species, with a particular emphasis on *H. Perforatum*. Investigations on *Hypericum dogonbadanicum* Assadi are relatively limited. Hence, the present study aims to explore the potential cytotoxic effects of *H. dogonbadanicum*, a plant species with a limited distribution, for the first time. Considering that ecological factors, such as altitude and growth conditions, can significantly influence the chemical composition even within different varieties of the same species, it is crucial to assess the cytotoxic properties of this unique plant. Therefore, the cytotoxicity of hydroalcoholic extract and EO from this valuable endemic species will be evaluated. The focus will be on evaluating its potential to induce apoptosis and examining any changes in the expression of *caspase-3* and *caspase-8* genes.

MATERIALS AND METHODS

Plant collection and preparation of EO and extract

The flowering aerial parts of *H. dogonbadanicum* were collected from southwestern Iran, Kohgiluyeh and Boyer-Ahmad province, 10 km east of Dogonbadan (30º 28.31' N, 50º 47.91' E), at 1600 m. A voucher specimen is deposited at IAUH (No. IAUH-12139, Identified by Iraj Mehregan).

The hydroalcoholic extract of the plant was prepared by employing the maceration technique.[25] For this reason, 30 grams of the dried twig powder was added to a glass jar containing 500 mL of 80% ethanol and placed in a shaker incubator (Innova 42) at 40ºC (100 rpm/24 hr). All organic solvents were Analytical Reagents (AR) grade chemicals obtained from Merck Chemical Inc. (Darmstadt, Germany). The extract was filtered through Whatman filter paper and concentrated by a rotary device (Heidolph Laboratory Rotary Evaporator, WB eco, Germany) at 4ºC temperature, 150-180 atm at 40 rpm. The EO was extracted from *H. dogonbadanicum* and hydro-distilled in a Clevenger-type apparatus for 4 hr. The collected EO was dried over anhydrous Na_2SO_4 and stored in a sealed vial in a refrigerator until it was required.[22]

HPLC evaluation of hypericin

Before the HPLC analysis, 0.3 g of the dried ethanolic extract was dissolved in a 4 mL solvent and filtered through a 0.45 µM filter membrane and then, 1 µL of ethanolic extract was injected into the device. Liquid chromatographic analysis of hypericin was performed on a High-Performance Liquid Chromatography (HPLC) system (KNAUER Company, HPLC PUMP K-1001). Hypericin was eluted isocratically, at 30ºC, on a reversed-phase Hypersil C18 column (Length×ID:250×4.6 mM), (Eurosphere 100-5) protected by a guard column of the same material and quantified by fluorescence detection at 322/593 nm (ex/em). The mobile phase was prepared weekly by mixing 95 volumes of methanol with 5 vol. of phosphate buffer solution (pH 2.2). For the preparation of the buffer solution, 2.5 g of KH_2PO_4 was dissolved in 950 mL double distilled water, adjusted to a pH of 2.2 with concentrated phosphoric acid and filled up to 1000 mL with double distilled water. The mobile phase was filtered before use, through a 0.45-µM nitrocellulose membrane and it was delivered isocratically at a flow rate of 1 mL/min.[26]

Gas Chromatography-Mass Spectrometry (GC/MS) of the EO

The Agilent 7890A/5975C GC/MS system with DB-5 fused silica column analyzed the EO composition (30 m×0.25 mM, i.d., film thickness 0.25 µM-ISO15303). The oven temperature program used for the experiment was as follows: The initial temperature of 60ºC was quickly raised to 220ºC at a rate of 3ºC per minute. It was then further increased to 260ºC at a rate of 20ºC per minute and held at this temperature for 5 min. Both the temperature of the injector and transfer line were maintained constant at 260ºC and 280ºC respectively. The carrier gas used was helium with a linear velocity of 30.6 cm/s and a split ratio of 1:100. The ionization energy used was 70 eV, the scan time was set to 1 sec and the mass range was from 40 to 300 a.m.u. The EO was also analyzed by GC, using an Agilent model 7890A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25 mM i.d., film thickness 0.25 µM-ISIRI-6117). The oven temperature programming was as follows: The initial temperature of 60ºC immediately increased to 220ºC at a rate of 3ºC/min and subsequently, the temperature rose to 260ºC at a rate of 20ºC/min and held at this temperature for 10 min. The detector (FID) and injector temperature were 280ºC. Nitrogen was used as a carrier gas with a linear velocity of 0.7 mL/min. Quantification data was obtained from GC-FID area percentages without the use of correction factors.[27]

Cell culture and reagents

The A549 cell line, which represents human lung epithelial-like cells, the AGS line, which represents human adenocarcinoma gastric cells and HDF (as a normal cell), were purchased from the Iranian Biological Resource Center (IBRC). Dulbecco's Modified Eagle's Medium (DMEM) was used for A549 cells, RPMI-1640 medium for AGS cells and Fibroblast growth medium

PCS-201-030 for HDF cells. Additional materials including FBS, trypsin-EDTA, glutamine and Dulbecco's Phosphate-Buffered Saline (D-PBS) were purchased from Gibco by Life Technologies, South America. The cell culture medium was supplemented with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin (BIO-IDEA, Iran) and 2 mM glutamine. The cultures were maintained at 37°C in a humidified atmosphere containing 5% v/v carbon dioxide. The adherent cells were sub-cultured every 3-4 days.

MTT cytotoxicity assay

MTT obtained from Sigma Aldrich Company (St Louis, MO, USA) was used to assess the cytotoxicity of the derived EO and extract of *Hypericum dogonbadanicum* on both A549 and AGS cancer cell lines and HDF as a normal cell. MTT assays were performed based on the standard protocol.[28,29] For this aim, 10^4 cells were distributed in 96 well-plates and kept in a 37°C incubator for 24 hr. Then, the culture medium of each well was slowly removed and afterward, cells were treated with different serial concentrations of plant extract and EO of 3.12, 6.25, 12.5, 25, 50, 100, 200 µg/mL and 50, 100, 200, 400 and 600 µg/mL, respectively. To measure survival 24, 48 and 72 hr after treatment, the plates were removed from the incubator separately and, 50 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hr. The MTT dye was then depleted and 50 µL of DMSO was added to each well and the plate was wrapped in aluminum foil, then incubated for about an hour in the incubator to dissolve the purple crystals from the MTT reduction and finally, the absorption of each sample in the wavelength of 570 nm was measured with an ELISA reader (Srcituro BiiketoiB). The resulting purple color represents the ratio of living cells in each well. The formula used to estimate cell viability was as follows:

Cell survival rate=100%×(mean control light absorption/mean sample light absorption)

Apoptosis detection

The apoptosis rate was quantitatively measured by Annexin V-FITC (Fluorescein isothiocyanate) Apoptosis Detection kit, using the manufacturer's protocol (BD Biosciences, Waltham, MA). Concisely, 2×10^5 density of cell lines, were treated with the IC_{50} value of each extract and EO and non-treated cell lines were detached using trypsin/EDTA and separated by centrifugation for 5 min at 112 g. The cells were washed with cold PBS and resuspended in 1-2 mL 1X AVBB (1×10^6 cells/mL) and centrifuged. Thereafter, the pellets were resuspended in 100 µL of PI staining mix in AVBB and added 2-4 µL of Annexin V-FITC. This mix was incubated for 15 min at room temperature and at least 500 µL of AVBB was added while keeping samples on ice. The mentioned above cells were immediately analyzed by the Flow cytometer (Becton Dickinson FACSCanto II (BD FACSCanto II, BD Biosciences, Waltham, MA).[30,31]

RNAs extraction and cDNA preparation

Total RNAs were extracted from both A549 and AGS cell lines that were treated with IC_{50} concentration of each extract and EO, as well as from non-treated cell lines. The RNA extraction was performed employing RiboEX kits (Cat. No.302-001) from GeneALL Korea. The purity of the extracted RNAs was determined using a nanodrop device (Srcituro BiiketoiB).[32]

cDNAs were synthesized from the extracted RNAs using the cDNA Synthesis Kits (Cat. No. A101161) from Pars Tous Biotechnology, Iran, according to the manufacturer's protocol. The synthesized cDNAs were cooled on ice and 1 µL of RNase H was added to the tubes, followed by incubation for 20 min at 37°C. The resulting cDNA products were either stored at -20°C for later use or used immediately for real-time PCR experiments.

Expression of caspase-3 and caspase-8 genes by Q-PCR assay

The mRNA expression of both *caspase-3* (Location: 4q35.1) and *caspase-8* (Location: 2q.33.1) was evaluated employing quantitative PCR-Assay (Q-PCR) by Rotor-Gene 6000 QIAGEN real-time PCR detection system.[33] The PCR primers were designed using the Primer3 online bioinformatics software (http://bioinfo.ut.ee/primer3-0.4.0) in accordance with a standard protocol. The quality of the primers was checked using Beacon Designer and M-FOLD bioinformatics software (Table 1). The Q-PCR was performed applying YTA SYBR Green qPCR Master Mix 2X kit in a final volume of 25 µL including 12.5 µL of cyber green, 1 µL of cDNA, 1 µL of each primer (0.2 pmol/µL) and 9.5 µL of distilled water. PCR amplicons were performed in 40 cycles according to the following steps: initial denaturation at 95°C for 15 min and then, for each cycle for 15 sec at 94°C, primers annealing at 60°C for 1 min and 10 sec for the final expansion at 70°C. Melting curve analysis was accomplished for PCR amplification in the temperature range from 70°C to 95°C.[29,30] The GAPDH (Location: 12p13.31) served as a housekeeping gene. All experiments were done in triplicate. The take-off and amplification data were used

Table 1: Gene names, primer sequences and primer length used in the current study.

Genes	Forward primer	Reverse primer	Product length
GAPDH	CTCATTTCCTGGTATGACAACG	CTTCCTCTTGTGCTCTTGCT	122
CASP3	GCTCTGGTTTTCGGTGGGTG	AAGCACCAAATCCACACTCCT	185
CASP8	ATTCAGCAAAGGGGAGGAGT	TTCAAAGGTCGTGGTCAAAG	241

to obtain the reaction efficiency in REST 2009 software. After obtaining E, the following formula was used to calculate the fold change of each gene in Excel 2016.

$$Fold\ change = \frac{(\mathrm{E}\ target)^{\Delta Ct\ target\ (control-sample)}}{(E\ ref)^{\Delta Ct\ ref\ (control-sampel)}}$$

Data analysis

The average data from the three replicates of the MTT assay were normalized and the IC_{50} values were calculated. A two-way ANOVA was conducted to compare the IC_{50} values between different cell lines. Moreover, a one-way ANOVA test was performed to analyze the results of the apoptosis test. Tukey's test was then applied to assess the significance of differences among sample means (p-value<0.05). The analysis was conducted utilizing GraphPad Prism version 9.3.1 (GraphPad Software, Inc., San Diego, CA) and SPSS version 21 (SPSS Inc., Chicago, Illinois, USA). Furthermore, the REST 2009 software was applied to calculate the reaction efficiency and compare the mean of three replicates of gene expression. An independent sample two-way ANOVA was also employed to assess the significance of the mean ΔCTs between the control and treatment groups.

RESULTS

HPLC analysis of the crude ethanolic extract

The chromatograms related to the ethanolic extract of *H. dogonbadanicum* (with a retention time of 4.8 min) and the standard hypericin (with a retention time of 4.7 min) over a run time of 10 min are shown in Figure 1. HPLC analysis of the crude extract of this species demonstrates hypericin as an anthraquinonoid compound, determined in the retention time of 4.802 min and peak area of 1.33803%. Consequently, the ethanolic extract was found to contain a precise yield of 3.6 mg/kg hypericin (Figure 1).

GC/MS analysis

The results of GC/MS analysis of the EO led to the recognition of 15 different chemical compounds in the species. ß-pinene, with 47.16% parts present in the EO, determined as the major isolated constituent, followed by α-pinene (29.49%), limonene (6.28%), camphene (3.03%), n-tetracosane (2.13%), n-pentacosane (1.86%) and n-tricosane (1.57%) (Table 2, Figure 1).

The red and the blue color of the first chromatogram indicate the standard hypericin with a retention time of 4.7 min and the ethanolic extract containing hypericin with a retention time of 4.8 min respectively.

Cytotoxic activity of an extract and the EO

The cytotoxic effect of different concentrations of an extract and the EO after 24, 48 and 72 hr of exposure against AGS, A549 and HDF cells, were screened properly employing MTT assay (Figure 2). The results were expressed as half-maximal Inhibitory Concentrations (IC_{50}) for each cell line which is expressed as the concentration of an EO and extract that is necessary to destroy half of the malignant cells. Therefore, the lesser the IC_{50} value, the more cytotoxic EO and extract for malignant cells. Based on our results, the IC_{50} value for the A549 cell line after 72 hr of an extract treatment was 50 µg/mL. Whereas, this value was equal to 200 µg/mL after 72 hr of EO treatment for this cell line (Figures 2 A, B). On the other hand, the IC_{50} value for the AGS cell line after 48 hr

Table 2: Phytochemical compounds of the *H. dogonbadanicum* EO.

Compounds	RT (min)	Area % (GC)	KI
α-pinene	8.66	29.49	941
Camphene	9.24	3.03	958
ß-pinene	10.26	47.16	984
Myrcene	10.42	0.47	990
P-cymene	11.92	0.23	1020
Limonene	12.11	6.28	1032
1,8-cineole	12.20	0.35	1036
ɣ-terpinene	13.25	0.53	1063
Terpinolene	14.41	0.81	1091
linalool	14.91	0.24	1100
n-tricosane	56.06	1.57	2300
n-tetracosane	57.43	2.13	2400
n-pentacosane	58.98	1.86	2500
n-hexacosane	59.63	0.35	2600
n-heptacosane	60.79	1.38	2700

RT: Retention Time KI: Kovats Retention Index.

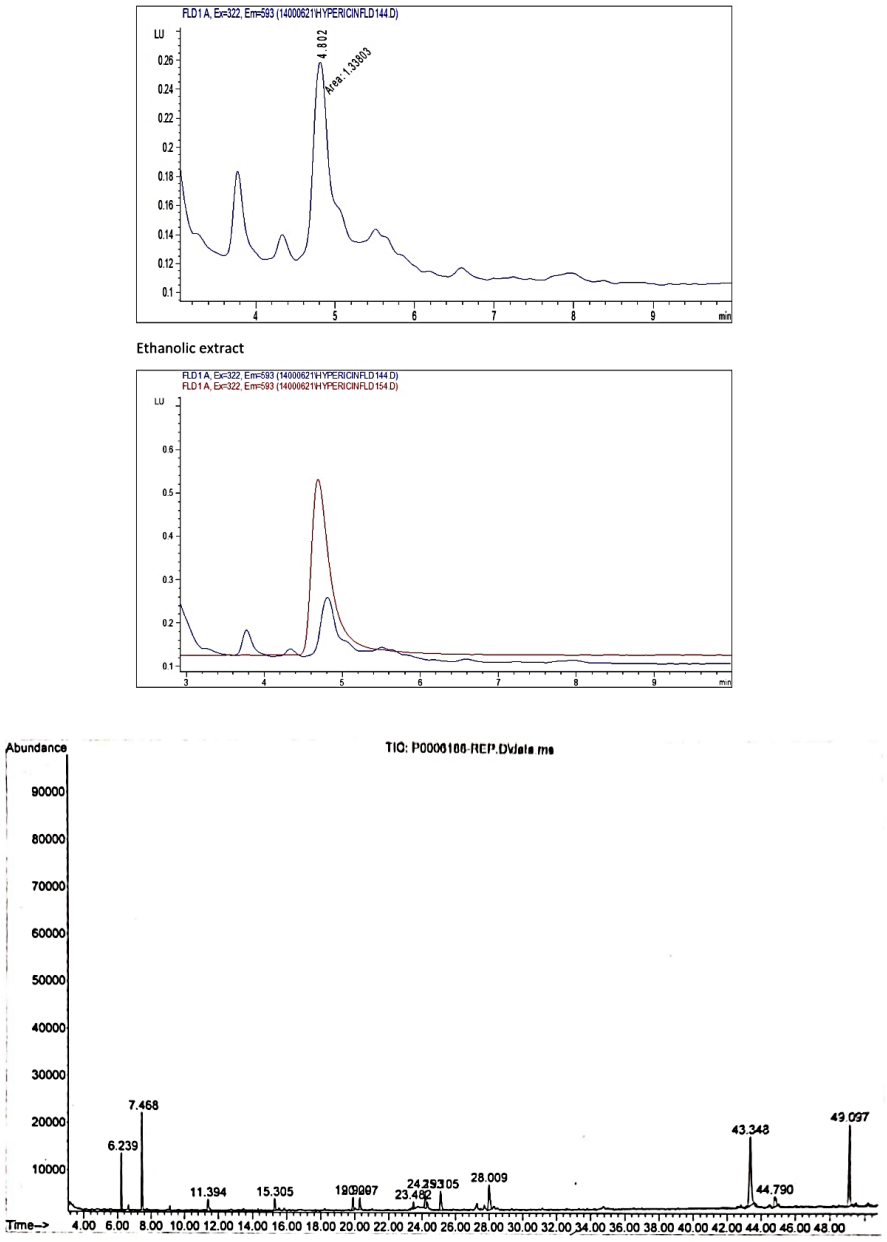

Figure 1: HPLC chromatogram of the extract and GC/MS chromatogram of the EO.

of extract exposure was equal to 200 µg/mL. However, this value was equal to 200 µg/mL when they were exposed to the EO after 72 hr (Figures 2 C, D). Although increasing the EO and extract concentrations of this species gives rise to an increasing number of cell deaths, it could not be the cause of 50% death of the normal HDF cells in each concentration (Figures 2 E, F). Moreover, the average percentage of the living cells in different concentrations was significant (p<0.05) compared to the control (Figure 3).

Extract and the EO induce apoptosis

A549 cells were exposed to IC_{50} concentration of an extract and the EO after 72 hr of treatment. The percentage of live, apoptotic and necrotic cells is shown in (Figures 4 A, B). The results demonstrated that, under the impression of an extract and EO the number of live cells decreased significantly (p<0.01). However, this reduction was especially affected by an extract rather than EO (Figure 4 B). This cell death pathway was an apoptosis death type more than necrosis. AGS cells were also exposed to the IC_{50} concentration of an extract and EO after 48 hr and 72 hr of

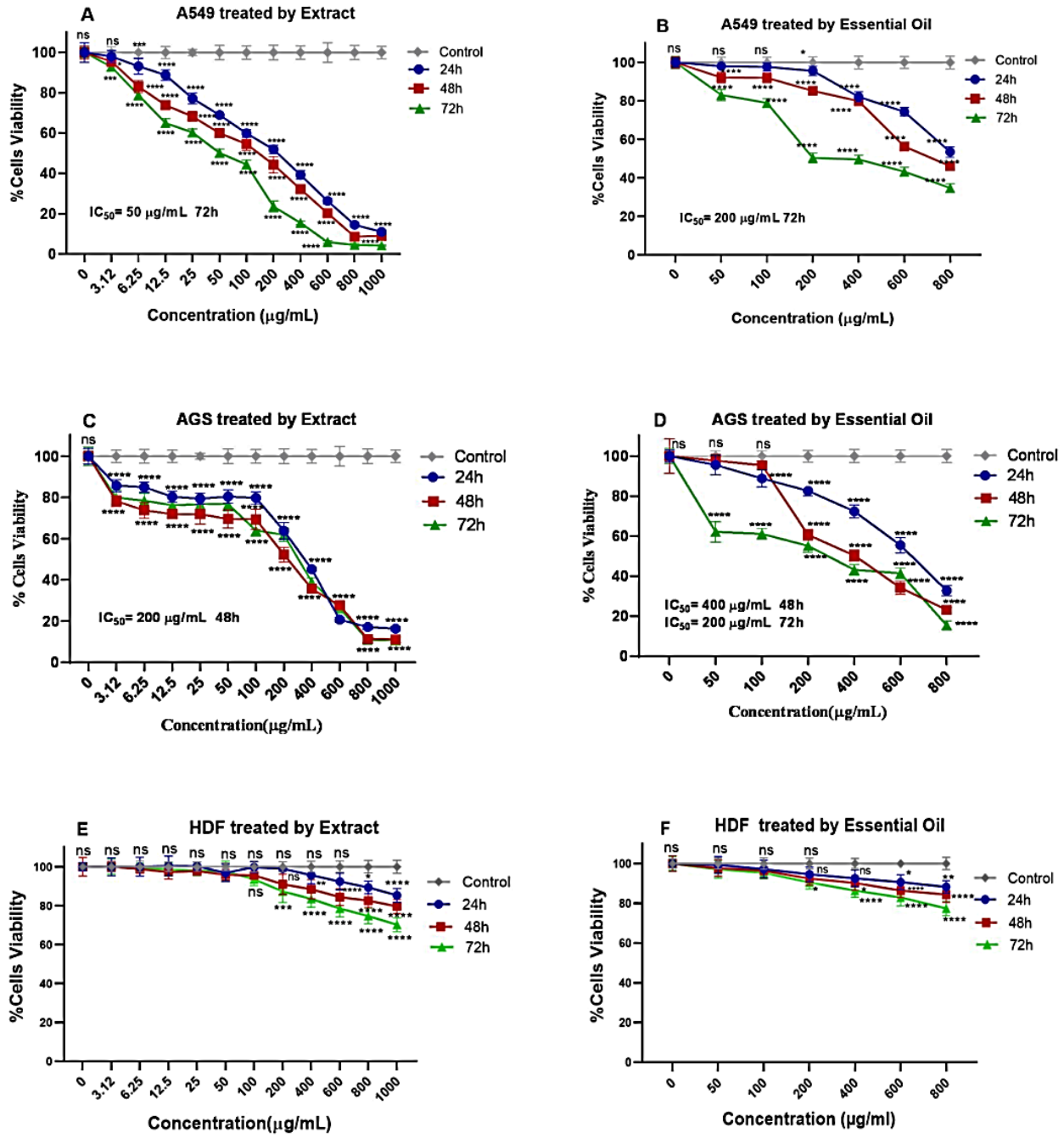

Figure 2: Anti-proliferative and inhibitory effects of different concentrations of the extract and the EO of *H. dogonbadanicum* on A549 (A, B), AGS (C, D) and (E, F) HDF cell lines during 24, 48- and 72 hr post-inoculation time and half-maximal inhibitory concentrations (IC_{50}) measured for each cell line. (Significant compared with control: ****p<0.0001, *** p<0.0001, ** p<0.01 * p<0.05 ns: non-significant difference).

treatment respectively. Both extract and EO had inducing effects on apoptosis and the number of live cells decreased significantly after treatment (Figures 4 A, B).

Gene expression analysis

In the present study, the expression level of both *caspase-3* and *caspase-8* was assessed for associations with IC_{50} concentration of derived extract and the EO from *H. dogonbadanicum* species. The results showed significant overexpression of both *caspase-3* and *caspase-8* associated with extract in the A549 cell line (p<0.01). Furthermore, in AGS cells, both *caspase-3* and *caspase-8* were significantly upregulated when treated with extract and EO (Figure 5). Extract treatment significantly increased the expression of both genes, especially *caspase-8*, in each cell line (p<0.01).

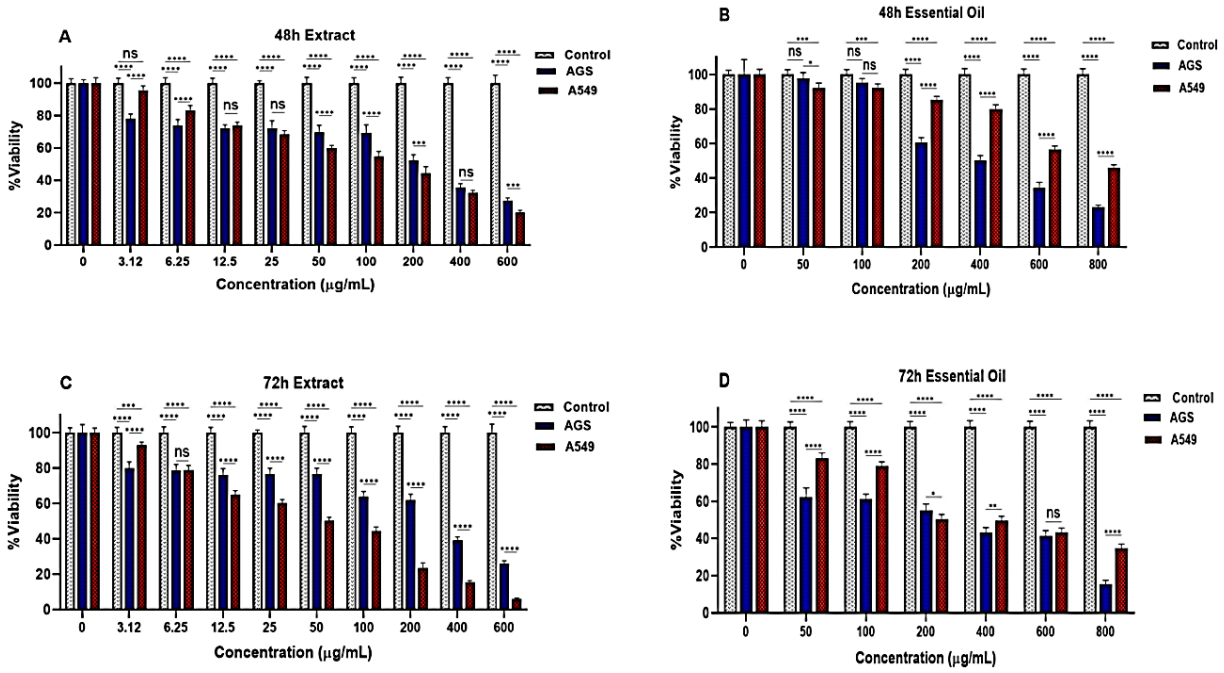

Figure 3: The mean survival rate of AGS (blue color) and A549 (red color) cell lines in response to different concentrations of an extract and the EO treatments. (A, C) Extract treatment (A) after 48 hr (C) after 72 hr (B, D) EO treatment (B) after 48 hr (D) after 72 hr (Significant compared with control: ****p<0.0001, *** p<0.0001, ** p<0.01 * p<0.05 ns: non-significant difference).

DISCUSSION

The role of natural antitumor agents in preventing cancer development is of great importance. These agents exhibit therapeutic effects by reducing the proliferation rate of carcinogenic cells, inducing apoptosis and inhibiting invasion and angiogenic factors. Moreover, their side effects are generally lower compared to other conventional anticancer drugs.[34] In this particular study, the antitumor effects of the EO and extract derived from *Hypericum dogonbadanicum* (Hypericaceae), were evaluated on gastric and lung cell lines. The chemical composition of the EO of *H. dogonbadanicum* has been previously reported. The main constituents of the EO were α-pinene (34.7%), β-pinene (32.1%), limonene (12.1%) and camphene (6.6%).[22] These compounds, particularly the three with the highest percentage, are considered to be the most abundant and effective in terms of their potential anticancer properties. Moreover, the most abundant compounds in the EOs were -pinene (54.3%), -pinene (12.0%) and p -cymene (11.0%).[23] The difference in the amounts of compounds reported by different studies may be attributed to variations in the harvest time and altitude of the plant samples.

In the present study, the *in vitro* cytotoxic effects, EO and extraction conditions of *H. dogonbadanicum* in both A549 and AGS cell lines were investigated for the first time. The concentration-response curve for an extract and EO for both A547 and AGS cells demonstrated the remarkable effects of dose-dependent phytocomponents during treatment (Figure 2). The inhibitory effect of EO was equal to 200 µL/mL for both AGS and A549 cell lines after 72 hr of treatment. Moreover, the cytotoxic effect of both extract and EO on the studied cancer cell lines was higher than that obtained for HDF as a normal cell representing that the EO and extract of the species had a low cytotoxic effect on the normal cell. The strong cytotoxicity of *Hypericum* Eos was observed against three human cell lines (A375, MDA-MB 231 and HCT116).[35] In the current research, the EO of one subspecies of *H. perforatum* showed moderate results against three human cancer cell lines (HeLa, HCT116 and U2OS).[36] The presence of the -pinene, - pinene as the main component in the EO could probably relate to its cytotoxic activity.

The comparison of the two cell lines revealed that the IC50 value for the A549 tumor cell line was lower than that reported for AGS after both 48 hr and 72 hr of exposure to the *H. dogonbadanicum* extract. In the present study, the precise yield of hypericin in the ethanolic extract is 3.6 mg/kg. However, the hypericin yield in the ethanolic extract of another species of *Hypericum* i.e., *H. perforatum* L. was 35 mg/kg by employing Soxhlet techniques.[37] Despite the lower amount of hypericin in *H. dogonbadanicum* in contrast with another related species, i.e., *H. Perforatum*, the antiproliferative activity of the plant extract on both AGS and A549 cell lines has been confirmed. This suggests that there may be other compounds present in *H. dogonbadanicum* with a significant role in exhibiting such properties. Future studies should focus on identifying and investigating these potential compounds. The pure composition of hypericin derived from the extract exhibited cytotoxic properties on AGS gastric cancer cell lines.[28] Additionally, the cytotoxic effects of Hypericin-mediated

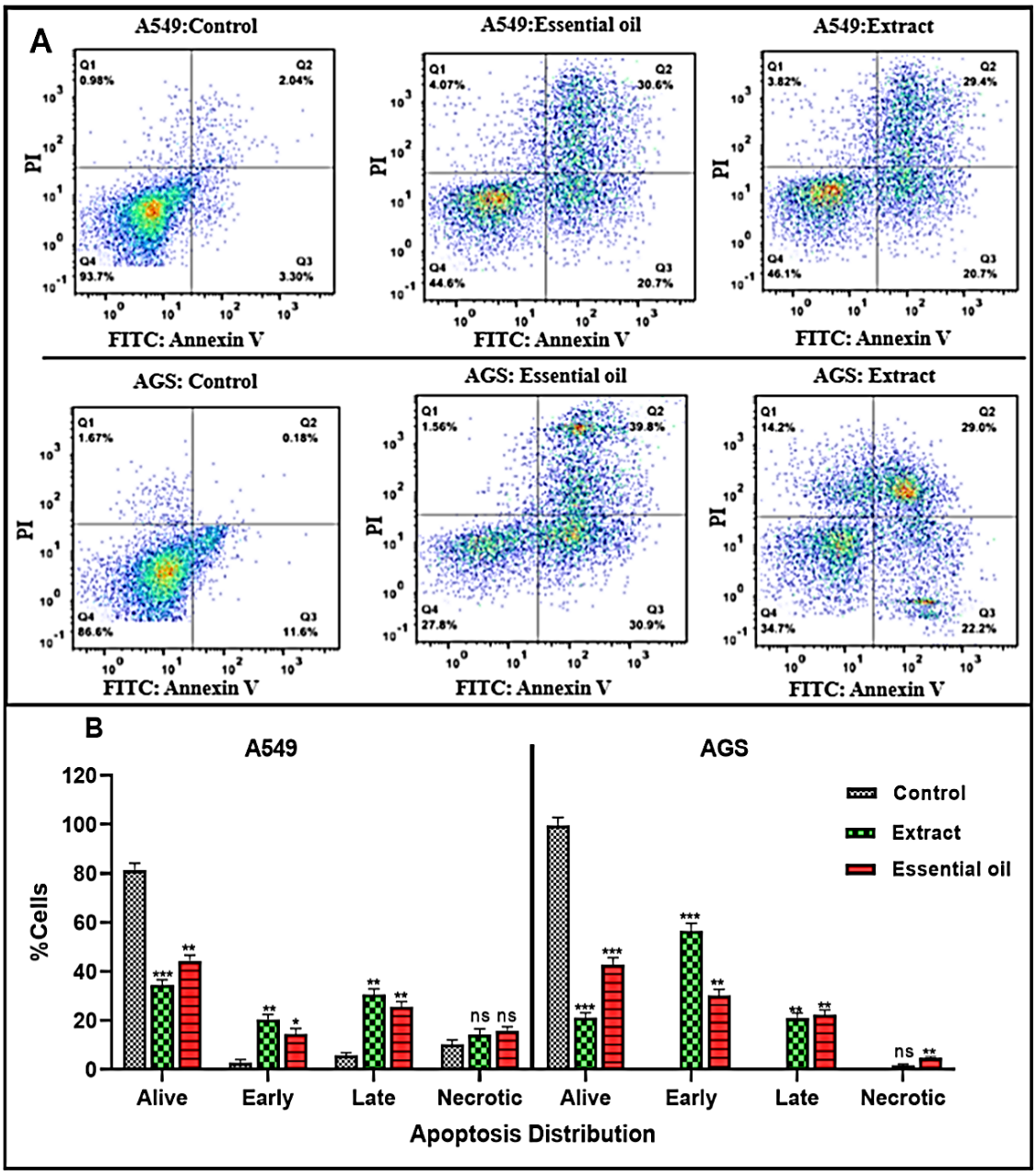

Figure 4: Annexin V-FITC analysis in both A549 and AGS cells (A) A549 cells treated with IC_{50} concentration of an extract and the EO after 72 hr of treatment (B) AGS cells treated with IC_{50} concentration of an extract and the EO after 48 and 72 hr respectively. The two cell lines had sensitivity to the extract and the EO. Q1=necrosis Q2=late apoptosis Q3=early Q4=viable cells (C) Bar chart comparison of cellular death type percentage in both A549 and AGS cell lines affected by an extract (green color) and the EO (red color). Control is shown in gray color. (Significant compared with control: *** p<0.0001, ** p<0.01 * p<0.05 ns: non-significant difference).

Photodynamic Treatment (HY-PDT) were shown to have the potential to both eliminate and control primary colon cancer. This further emphasizes the potential therapeutic applications of hypericin in cancer treatment.[38] Apoptosis, also known as programmed cell death, is a highly regulated process that plays a crucial role in maintaining tissue growth and homeostasis by eliminating aging, damaged and unwanted cells. The antitumor activity of hypericin, including its apoptotic and anti-metastatic effects, has been evaluated in various types of cancers in previous studies.[39-42] The results of the current study demonstrated the ability of the extract and EO of *H. dogonbadanicum* to induce apoptosis in both the studied cell lines (Figure 3). However, it was observed that the extract exhibited greater effectiveness in inducing cell apoptosis compared to the EO in the AGS cell line. The abnormal expression of caspase and/or activation of *caspase-3* and *caspase-8* is associated with different types of cancer.[11] *Caspase-3* overexpression and expressionless have been reported in varied human cancers.[43] Loss of *caspase-8* mRNA/protein

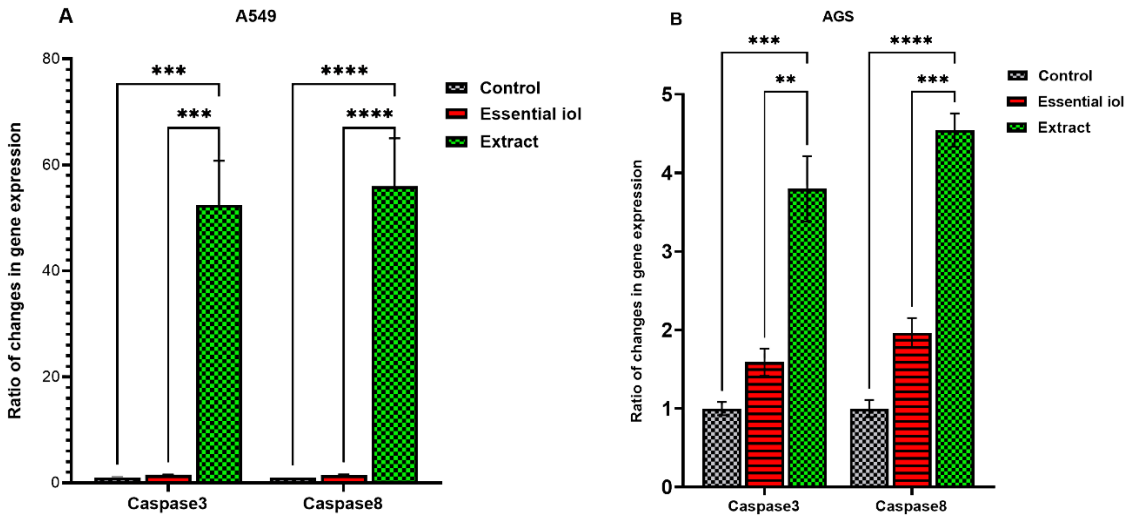

Figure 5: Expression pattern of *caspase-3* and *caspase-8* in both A549 and AGS cell lines under the influence of extract and the EO of *H. dogonbadanicum*. Gene expression diagrams show significant overexpression pattern in both *caspase*-3 and *caspase*-8 genes in two cell lines under the exposure of extract (A) upregulation of A549 cell line under the influence of extract separately (B) upregulation of AGS cell line under the impression of both the EO and extract of the plant (Significant compared with control: ****$p<0.0001$, *** $p<0.0001$, ** $p<0.01$ ns: non-significant difference).

expression has been demonstrated in high-grade small-cell lung cancer, neuroendocrine lung cancer and pediatric neuroblastoma. Previous studies have shown that *caspase-8* might act as a tumor suppressor in certain types of lung cancer and neuroblastoma.[44] In the present study, the results of data analysis revealed a significant increase in *caspases-3* and *caspase-8* gene expression in both A549 and AGS cell lines while exposed to the plant extract and the AGS cell line showed better response to the plant components compared to the A549 cell line. However, under the impression of the EO of the plant, significant upregulation was only observed in AGS cells. Lung, gastric and other malignancies often have dysregulated *caspase-3* and *caspase-8* expression patterns, leading to inequality between their apoptotic and non-apoptotic roles within the tumor and its microenvironment.[45] Hyperforin increases *caspase*-9 and *caspase*-3 activity in U937 leukemia cells and also raises caspase-8 and *caspase*-3 activity in K562 cells.[42]

According to the recent study conducted by Brankiewicz *et al.*, (2023), extracts of *H. perforatum* sourced from various origins, which contain differing levels of bioactive phenolic components and hyperforin, exhibit varying levels of activity against malignant cells in both normoxic and hypoxic conditions. The synergistic effects of *H. perforatum* extract compounds and pure hyperforin in its salt form play a critical role in the viability, apoptosis and oxidative stress of melanoma cells. Furthermore, varying gene expression levels were noted based on the dosage and cell line under these two mentioned conditions. The findings indicate a significant complexity in the mechanisms of action of the extracts used, which differ according to their composition. Additionally, they indicate that hyperforin and its derivatives, as individual molecules, can exhibit distinct behaviors by activating different signaling pathways within the cell. They discovered that the composition of extracts, which contributes to their unique properties, may play a vital role in the selection of plants for therapeutic applications.[46]

CONCLUSION

In conclusion, the findings of this study suggest that the extract and EO of *H. dogonbadanicum* display potential as an effective apoptotic inducer for cancer cell lines (A549 and AGS). This effect is achieved by targeting and upregulating the expression of *caspase-3* and *caspase-8* genes, primarily attributed to the presence of hypericin. Nevertheless, further research is warranted to explore the therapeutic applications of this plant species.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FBS: Fetal bovine serum; **MTT:** (3-4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **EO:** Essential oil; **AVBB:** Annexin V Binding Buffer; **HDF:** Human Dermal Fibroblasts.

SUMMARY

Hypericum dogonbadanicum is regarded as an Iranian endemic medicinal herb due to its outstanding therapeutic properties. In the present study, we demonstrated that extract and EO can induce Apoptosis in Gastric (AGS) and lung (A549) cancer cell lines by targeting and increasing *caspase-3* and *caspase-8* gene expression levels. However, the cytotoxic effect of both extract and EO on HDF was lower than those obtained for two mentioned cancer cell lines showing that the EO and extract of the species had a low cytotoxic effect on the normal cell.

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