MS EDITIONS Blacpma

BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Cytotoxic effect of hydroalcoholic extract of *Cota tinctoria* (L.) J.Gay on AGS and Hep-G2 cancer cell lines

[Efecto citotóxico del extracto hidroalcohólico de *Cota tinctoria* (L.) J. Gay sobre líneas celulares de cáncer AGS y Hep-G2]

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Section Biological activity

Received: 28 September 2020
Accepted: 27 October 2020
Accepted corrected: 3 January 2021
Published: 30 January 2022

Citation:
Shamloo S, Marandi SJ, Tajadod G, Majd A, Rahimi R
Cytotoxic effect of hydroalcoholic extract of *Cota tinctoria* (L.) J. Gay on AGS and Hep-G2 cancer cell lines
Bol Latinoam Caribe Plant Med Aromat
21 (1): 108 - 122 (2022).
https://doi.org/10.37360/blacpma.22.21.1.07

Abstract: *Cota tinctoria* is a medicinal plant which has been used for management of cancer in folk medicine of various regions. The aim of present study is to investigate cytotoxic activity of different concentrations of hydroalcoholic extract of *C. tinctoria* flowers on gastric (AGS) and liver (Hep-G2) cancer cell lines as well as Human Natural GUM fibroblast (HUGU) cells. Cell mortality rates were examined after 24, 48 and 72 h incubations using the MTT assay. IC_{50} of extract on AGS cells after 24, 48 and 72h was 1.46, 1.29 and 1.14 µg/mL respectively. The extract demonstrated IC_{50} of 5.15, 3.92 and 2.89 µg/mL on Hep-G2 cells after 24, 48 and 72 h respectively. No cytotoxic effect was detected on HUGU (Human Natural GUM fibroblast) cells. *C. tinctoria* seems to have a promising potential to be considered as a source for anticancer drug discovery. However, more experimental and clinical studies are required.

Keywords: Antitumor; Gastric cancer; Hepatic cancer; *Cota tinctoria*; *Anthemis tinctoria*.

Resumen: *Cota tinctoria* es una planta medicinal que se ha utilizado para el tratamiento del cáncer en la medicina popular de varias regiones. El objetivo del presente estudio es investigar la actividad citotóxica de diferentes concentraciones de extracto hidroalcohólico de flores de *C. tinctoria* en líneas celulares de cáncer gástrico (AGS) e hígado (Hep-G2), así como en células de fibroblasto GUM humano natural (HUGU). Se examinaron las tasas de mortalidad celular después de incubaciones de 24, 48 y 72 h utilizando el ensayo MTT. La CI_{50} del extracto en células AGS después de 24, 48 y 72 h fue de 1,46; 1,29 y 1,14 µg respectivamente. El extracto demostró una CI_{50} de 5,15, 3,92 y 2,89 µg/mL en células Hep-G2 después de 24, 48 y 72 h, respectivamente. No se detectó ningún efecto citotóxico en las células HUGU (fibroblasto GUM humano natural). *C. tinctoria* parece tener un potencial prometedor para ser considerada como una fuente de descubrimiento de fármacos contra el cáncer. Sin embargo, se requieren más estudios experimentales y clínicos.

Palabras clave: Antitumoral; Cáncer gástrico; Cáncer de hígado; *Cota tinctoria*; *Anthemis tinctoria*.

LIST OF ABBREVIATIONS
- Dimethyl sulfoxide (DMSO)
- Human gastric adenocarcinoma (AGS)
- Human Gum Fibroblast (HUGU)
- Human hepatocellular carcinoma (Hep-G2)
- (3-[4,5-dimethyethiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT)
- Phosphate buffered saline (PBS)
- The half maximal inhibitory concentration (IC_{50})

INTRODUCTION

Over the last decades, cancer has been the most challenging disease to cure and remains the second leading cause of death worldwide (Ozols *et al*., 2008). A report released by the World Health Organization (WHO), showed that estimated 12.7 million people were diagnosed with cancer globally and about 7.6 million people died of it in 2088 (Zong *et al*., 2012). As estimated in this report, more than 21 million new cancer cases and 13 million deaths are expected by 2030. Although cancer accounts for around 13% of all deaths in the world, more than 30% of cancer deaths can be prevented by modifying or avoiding key risk factors (World Health Organization, 2012). Chemotherapy as one of therapeutic option for cancer until recently still has the drawbacks of severe side effects and dose-limiting toxicity (Camp-Sorrell, 2000). The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body (Chabner *et al*., 2005). Therefore, the identification of non-toxic chemotherapeutic from herbal medicines remains to be an attractive goal to advance cancer treatments (Susanti *et al*., 2012). Herbal medicine represents traditional medicines such as a variety of efficacious plant extracts, which have been known to enhance healing of various diseases for thousands of years (Susanti *et al*., 2012). It has been recognized that utilization of herbal medicine may become potential treatments in the future (Fabricant & Farnsworth, 2001). This is evidenced by the practices of traditional medicine that have been well accepted and trusted by the public until these days (Hoareau & da Silva, 1999). Ideally any anticancer drug should have an acceptable therapeutic index, that is, the drug should exert a cytotoxic effect on malignant cells with minimal effect on normal cells (Echiburu-Chau *et al*., 2014). Indeed, numerous anticancer drugs in current use have been extracted from plants, including vinblastine, an alkaloid extracted from *Catharanthus riseus* that inhibits the assembly of the mitotic spindle microtubules (Makarov *et al*., 2007), and paclitaxel, an alkaloid extracted from *Txus brevifolia* whose activity stabilizes microtubules during cell division (Cisternino *et al*., 2003). Several other drugs extracted from plants have recently proven useful as anticancer therapies, including betolunic acid (Fulda., 2008), resveratrol (Garcia-Zepeda *et al*., 2013) and homoharringtotine (Zhou *et al*., 1995), among others. Exploring natural products as novel chemopreventive and chemotherapeutic drugs in order target them to patients at high risk of developing cancer and metastasis can result in better prognosis (Rahimi *et al*., 2015). Mounting evidence suggest that phytochemicals derived from medicinal plants have demonstrated anticancer activity mediated by induction of apoptosis, as well as delaying metastasis (Da rocha *et al*., 2001; Gordaliza., 2007; Azimi *et al*., 2015)

The genus *cota* J. Gay (Asteraceae, Anthemideae) consists of 49 species (Including 63 taxa) worldwide. *Cota* J. Gay, which belongs to the tribe Anthemideae (Asteraceae), is represented by 63 taxa in the world and is mainly distributed in Europe, North Africa, and Central Asia. *Cota* was earlier classified as a section in the genus *Anthemis* L. *Anthemis* s.l. is divided into three sections, namely sect. *Anthemis* (29 species), sect. *Maruta* (Cass.) Griseb (six species) and sect. *Cota* (J. Gay) (15 species) (Oberprieler, 2001; Greuter *et al*., 2003; Lo Presti *et al*., 2010; Ozbek *et al*., 2016). *Cota tinctoria*, also known as golden marguerite, yellow chamomile, is a species of perennial flowering plant in the Asteraceae family (Franke, 2005).

The species of the *Cota* (synonym *Anthemis*) genus are widely used in the pharmaceutic, cosmetic and food industries. The uses of the flowers of this genus are well-documented in antiseptics, as are healing herbs, the main components being natural flavonoids and essential oils. Its extracts are used to allay pain and irritation, clean wounds and ulcers, aid in prevention as well as therapy of irradiated skin injuries, and the treatment of cystitis and dental afflictions (Papaioannou *et al*., 2007). The plants of genus *Cota* are among less investigated species. In literatures, there is information that the flowers of the genus cota were used as antiseptic and healing herbs (Ozek *et al*., 2018).

Flavonoids and other phenolic compounds are commonly known as plant secondry metabolites that hold an aromatic ring bearing at least one hydroxyle groups. (Kumar & Pandey, 2013; Ahmed *et al*., 2016). These phytochemical substances are presented in nutrients and herbal medicines, both

flavonoids and many other phenolic components have been reported on their effective antioxidants, anticancer, antibacterial, cardioprotective agents, anti-inflammation, immne system promoting and interesting candidate for pharmaceutical and medical application. (Kumar & Pandey, 2013; Chen et al., 2015; Andereu et al., 2018; Meng et al., 2018)

However, herbal medicine has not been fully accepted as cancer therapeutics due to lack of experimental and clinical studies about their efficacy and safety (Buchanan *et al*., 2005) In vitro study to evaluate their efficacy and safety is necessary prior to the further tests either in vivo study clinical trial.

The aim of this study is to evaluate the cytotoxic activities of hydroalcoholic extract of *C. tinctoria* against Human gastric adenocarcinoma (AGS) and Human hepatocellular carcinoma (Hep-G2) cancer cell lines and Human Natural GUM fibroblast (HUGU) cell line.

MATERIALS AND METHODS

Plant material

C. tinctoria was collected in June (2017) from North Iran (Gachsar), GPS 36452018N, 5119128E. The plant was confirmed by Dolatyari & talebi in Iranian Biological Resource Center (IBRC) Herbarium and a voucher specimen (IBRC NO.P1013000) deposited in the (IBRC) Herbarium. The flowers were isolated and dried in the shade, and stored at room temperature for 14 days.

Extract preparation

For preparation of *C. tinctoria* hydroalcoholic extract, 200 g of dried flowers were powdered and extracted by maceration at room temperature (24 ± 3°C) using 600 mL ethanol (80%) as solvent for 72 h. The extract was filtered and evaporated at reduced pressure to yield residues of about 16.4 g on the basis of dry plant material. The extract was stored at -18°C for the experimental procedure.

Determination of total phenolic content

Total phenolic content of extract was determined using folin-ciocalteu reagent (Memariani *et al*., 2017). For this purpose, distilled water was added extract (100 mg) up to 10 mL The mixture was sonicated for 5 min and then filtered. Filtrate (1 mL) was mixed with folin-ciocalteu reagent (1.5 mL) which previously diluted 10-fold with distilled water, and allowed to stand at room temperature for 5 min. 1.5 mL of bicarbonate solution (60 g/L) was added to the mixture. After staining for 90 min at room temperature, the absorbance was measured at 725 nm using a UV-visible spectrophotometer (GBC, Cintra 40). The same procedure was repeated for the gallic acid standard solution. The gallic acid solution was prepared at concentrations of 50 to 200 μg/ml. To achieve phenolic concentration, the gallic acid calibration curve based on the detected absorbance was construed (Figure No. 1). All tests were carried out in triplicate and the results were expressed as gallic acid equivalents (mg GAE/g dry weight).

Determination of total flavonoid content

Total flavonoid content of extract was determined by the aluminum chloride colorimetric method (Memariani *et al*., 2017). For this purpose, distilled water was added to 200 mg extract up to 10 mL. The mixture was sonicated for 5 min and then filtered. Concisely, 1 mL of filtrate was added to 10 mL volumetric flask containing 4 mL of double distilled water. 0.3 mL $NaNO_2$ (5%) was added to the flask and 5 min later 0.3 mL $AlCl_3$ (10%) was added. After 6 min, 2 mL NaOH (1 M) was added and the total volume was made up to 10 mL and the flask contents were thoroughly mixed. The absorbance level was measured versus blank at 510 nm (GBC, Cintra 40). The same procedure was performed for the quercetin standard solution. The quercetin solution was prepared at concentrations of 5 to 80 μg/ml and the calibration line was construed (Figure No. 1). The calibration curve was plotted using standard quercetin. Total flavonoid contents were represented as mg catechin equivalents (CE) per one gram dry extract according to the catechin standard solutions.

Cell culture

AGS (Human gastric adenocarcinoma), Hep-G2 (Human hepatocellular carcinoma) and HUGU (Human GUM fibroblast) cell lines were provided by the Iranian Biological Resource Center (IBRC) (Tehran, Iran).

Maintenance of human cell lines

For culturing AGS, Hep-G2 and HUGU, the culture medium DMEM (Dulbecco's Modified Eagle Medium) containing 10% Fetal Bovine Serum (FBS) and 2 mL glutamine was used. These were cultured under standard incubation conditions (temperature 37°C, CO_2 5%, humidity 95%). After three passages, the cells were used for later processing, and cell count and number of living cells were counted using the hemocytometer with Trypan Blue (Cheryl *et al*., 2004; Selenius *et al*., 2019).

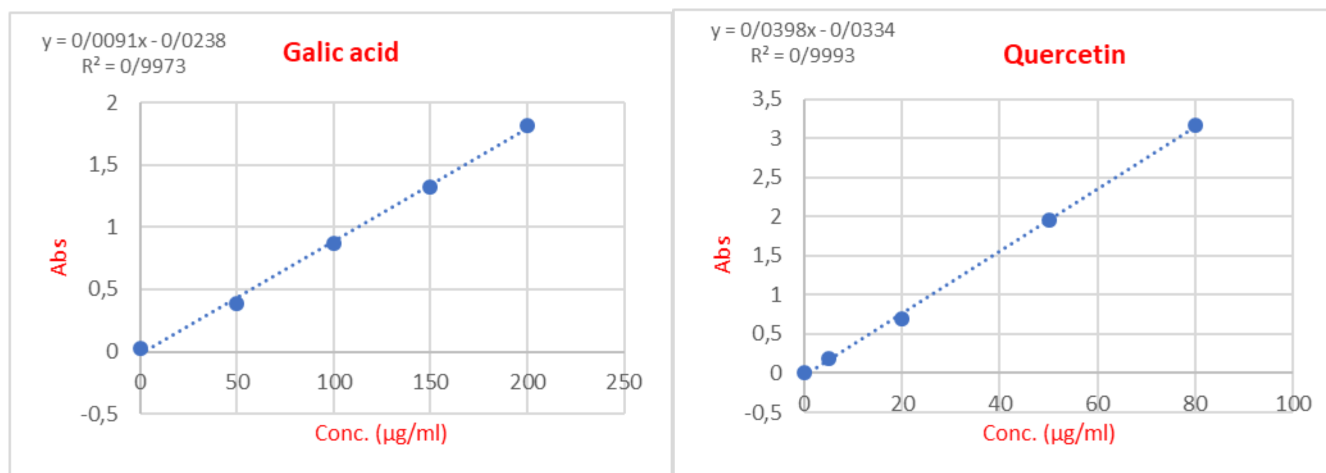

Figure No. 1
Calibration curves for gallic acid and quercetin standards to determine total phenol and flavonoid contents of hydrogel respectively

MTT- based cytotoxic assay
Cytotoxicity studies were performed using the MTT (3-[4,5-dimethyethiazole-2-yl]-2,5-diphenyltetrazo-lium bromide) assay (Mosmann *et al*., 1983).

After covering the flask bed with cells, the cell layer sticking to the flask bottom was separated using trypsin. After being transferred to sterile test tubes, they were centrifuged at 1200 rpm for 5 min. Cells were then suspended using a Pasteur pipette in new culture medium. After counting, 200 µL of cells (5×10^4 cell per mL media) were seeded in 96-well plates and incubated at 37°C for 24 h (5% CO_2 humidified). After the required time, the supernatant was removed slowly and carefully. Hydroalcoholic extracts of *C. tinctoria* were added to wells at concentrations of 0.09, 0.19, 0.38, 0.75, 1.25, 2.5, 5, 10, and 20 µg/mL

Serum containing media without extract were added to control wells. Plates were incubated for 24, 48, and 72 h. After the incubation period, plates were removed from the incubator. The supernatant from each well was completely removed by the sampler and cells were washed with 100 µL PBS Phosphate buffered saline before 80 µL of media and 20 µL yellow MTT solution was added. The plates were incubated at 37ºC for 3 h. After the required time, first the supernatant was completely removed and each well washed with 100 µL PBS, and 100 µL DMSO was added to dissolve formazan crystal which were quantified by reading the absorbance at 570 nm on a microplate reader (Anthos Lab Tech Instruments, Austria) and used as ameasure of cell viability. Six wells were assayed for each condition, and mean as standard deviations were determined using Microsoft Exel. The IC_{50} values (concentration inducing a 50% inhibition of cell growth) were calculated from the equation of the logarithmic line determined by fitting the best line to the curve formed from the data by using Sigma plot 10.0. The IC_{50} value was obtained from the equation y=50 (50% value). In all experiments, the final concentration of DMSO did not exceed 1% (v/v), a concentration that was nontoxic to the cells and cells with no treatment and methotrexate (Sigma, USA) treatment were used as a negative and positive control, respectively.

In order to convert the amount of light absorption into a percentage of live cells, the following formula was used and live % of cells after 24, 48, and72 h was computed.

Biological ability % = OD Test (optical absorption of extract/Optical absorption of solvent)×100

Statistical analysis
Data were analyzed using SPSS software via the one-way ANOVA method and mean comparison was done with the Tukey method. P-values less than 0.05 were considered to be statistically significant.

Cell Morphological Analysis method

Cell morphology assessment confluent cultures of AGS and Hep-G2 and HUGU cells were treated with the hydroalcoholic extract of flowers of *cota tinctoria* L. for 24, 48 and 72 h and photographed at the refrence points with a phase-contrast microscope (OLYMPUS CKX41, Olympus, London.UK) and the photos were taken with digital camera (Canon CCD 2272×1702, Argentina).

RESULTS

Total phenolic and total flavonoid content

The total phenolic content of extract was 1168.42 ± 12.68 mg GAE/g respectively. The total flavonoid content of each tablet was 582.15 ± 7.38 mg of CE/g of dry extract respectively by reference to the related standard curves.

Evaluation of cytotoxic activity

Results showed that in a 24 h incubation period, viability in AGS and Hep-G2 cell lines was reduced with increasing dose of hydroalcoholic extract of *C. tinctoria*. The viability percentage in AGS cancer cells was reduced from 86% in 0.09 µg/mL concentration to 20.2% in 20 µg/mL. In Hep-G2 cancer cells viability was reduced from 99.9% in 0.09 µg/mL concentration to 25.8% in 20 µg/mL. These changes were statistically significant ($p \leq 0.05$) (Figure No. 2).

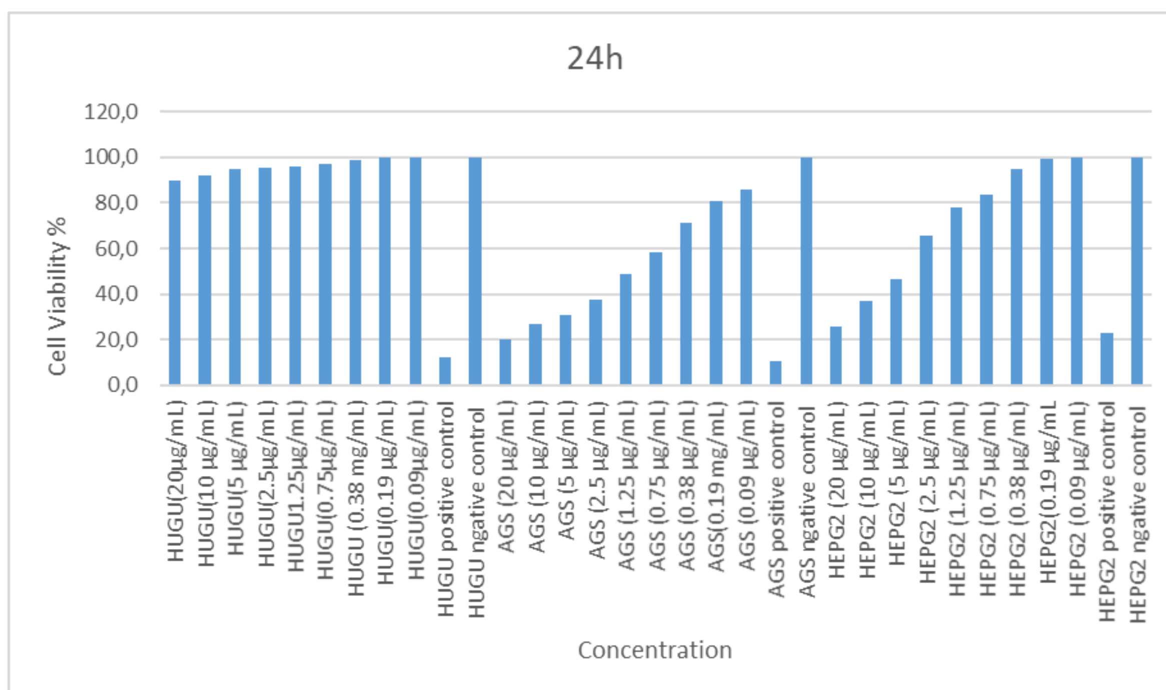

Figure No. 2
Cytotoxic activity of *C. tictoria* Hydroalcoholic extract against AGS, Hep-G2 and HUGU cell in different dosage after 24

In the 48 h incubation, dose-dependent reduction of viability was observed. The percentage of viability was decreased in AGS cancer cells from 82% in 0.09 µg/mL concentration to 19.8% in a concentration of 20 µg /mL. In Hep-G2 cancer cells viability decreased from 98.8% in 0.09 µg/mL concentration to 24.4% in concentration of 20 µg/mL. This was statistically significant ($p \leq 0.05$) (Figure No. 3).

In the 72 h incubation, the reduction of viability percent was also observed in AGS cancer cells from 79.7% in a concentration of 0.09 µg/mL to 16.3% in 20 µg/mL and in Hep-G2 cancer cells it also decreased from 97.7% in 0.09 µg/mL concentration to 20.1% in 20 µg/mL. This was statistically significant ($p \leq 0.05$) (Figure No. 4).

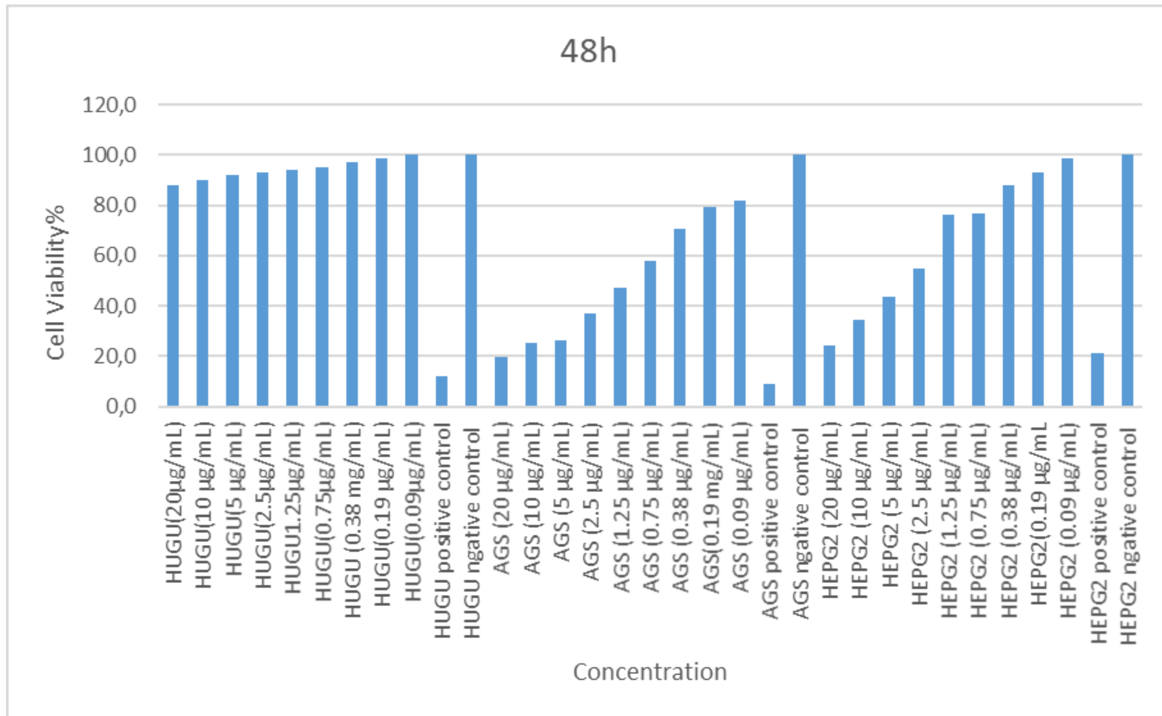

Figure No. 3
Cytotoxic activity of *C. tictoria* Hydroalcoholic extract against AGS, Hep-G2 and HUGU cell in different dosage after 48 h

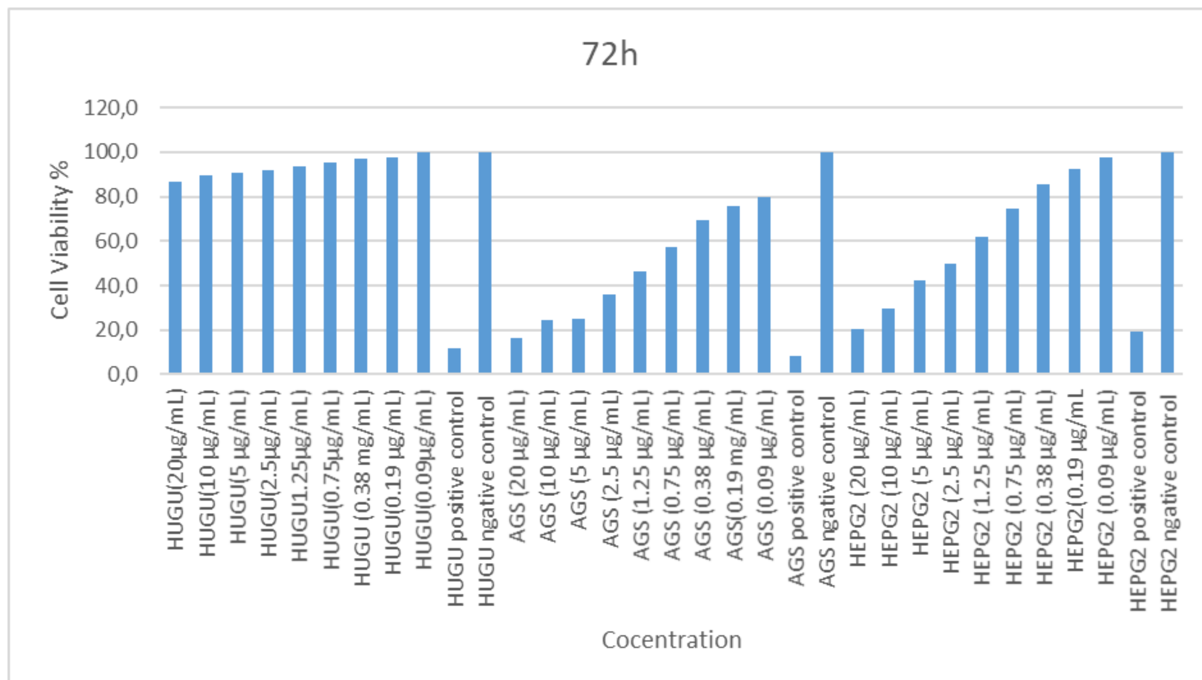

Figure No. 4
Cytotoxic activity of *C. tictoria* Hydroalcoholic extract against AGS, Hep-G2 and HUGU cell in different dosage after 72 h

The result of the MTT assay showed that hydroalcoholic extract of *C. tinctoria* had little effect on the HUGU cell line (Figures No. 2 & No. 4).

Cell morphological analysis

Findings for AGS cancer cell growth after 24, 48, and 72 h of treatment with hydroalcoholic extract of *C. tinctoria* (concentrations: 0.09 µg/mL, 0.19 µg/mL, 0.38 µg/mL, 0.75 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL (Figures No. 2 & No. 4). After exposure to a concentration of 20 µg/mL of extract, cells were distorted and their morphology changed, showing toxicity effects of hydroalcoholic extract of *C. tinctoria* on AGS cells (Figure No. 5C & No. 5D).

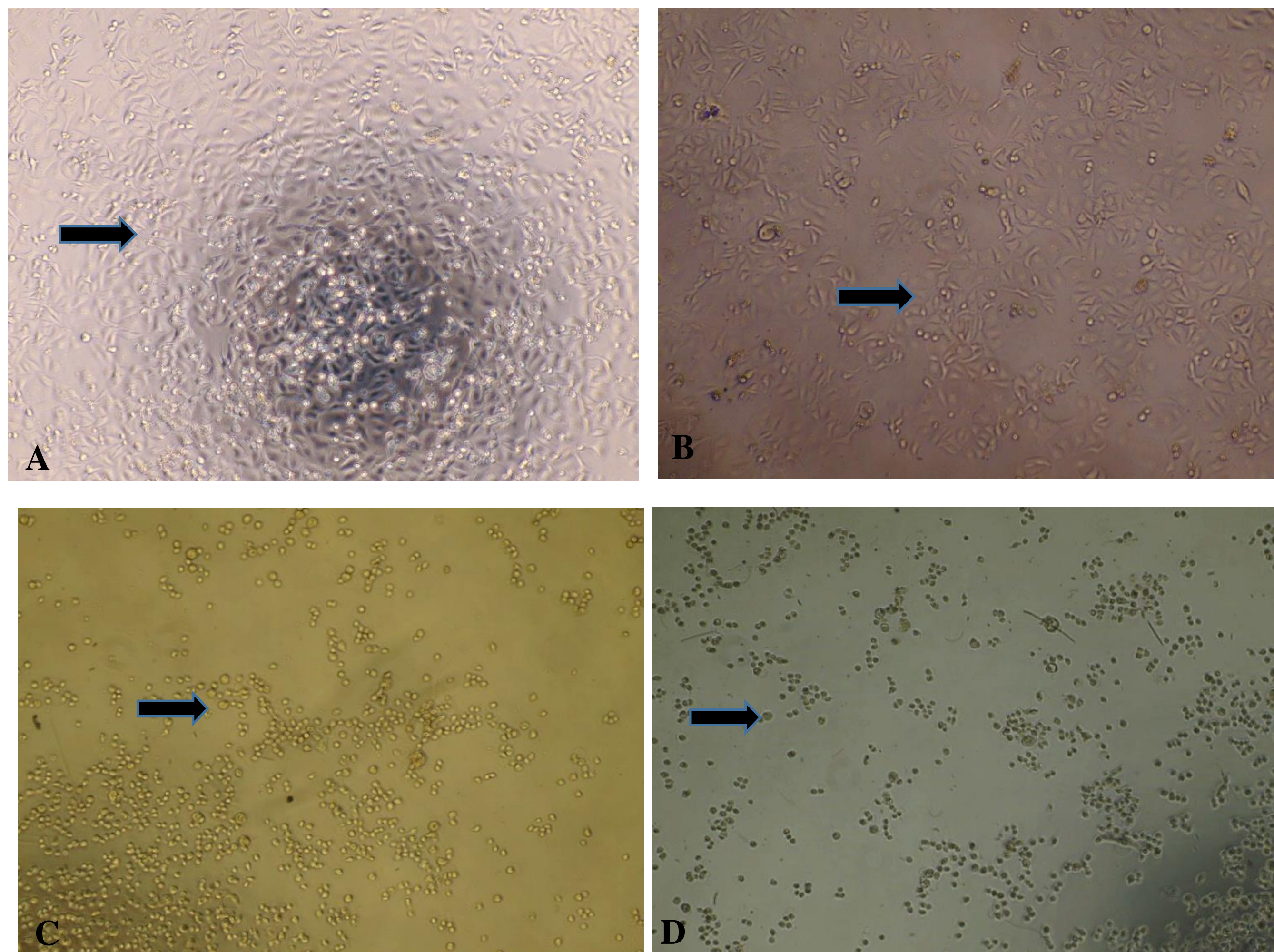

Figure No. 5
Morphological changes of AGS cancer cells (A): AGS cancer cell in the control group without treatment by hydroalcoholic extract of *C. tinctoria* (Negative control). (B): Positive control. (C): Morphological changes of AGS cancer cells after 48 h treatment with hydroalcoholic extract of *C. tinctoria* at a concentration of 20 µg/mL, (D): AGS cancer cells after 72 h treatment with hydroalcoholic extract of *C. tinctoria* at a concentration of 20 µg/mL. Phase contrast analysis evidenced that the cells treated with the sample at different concentration showing apoptotic bodies, cell shrinkage and reduced in number and size.

Hep-G2 cancer cell growth after 24, 48, and 72 h of treatment with hydroalcoholic extract of *C. tinctoria* (concentrations: 0.09 µg/mL, 0.19 µg/mL, 0.38 µg/mL, 0.75 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL) (Figure No. 2-4) was prevented in comparison to the control group (no treatment with hydroalcoholic extract of *C. tinctoria* (Figure No. 6A).

After exposure to a concentration of 20 µg/mL of extract, cells were distorted and their morphology was changed, showing toxicity effect of hydroalcoholic extract of *C. tinctoria* on Hep-G2 cells. (Figures No. 6C & No. 6D).

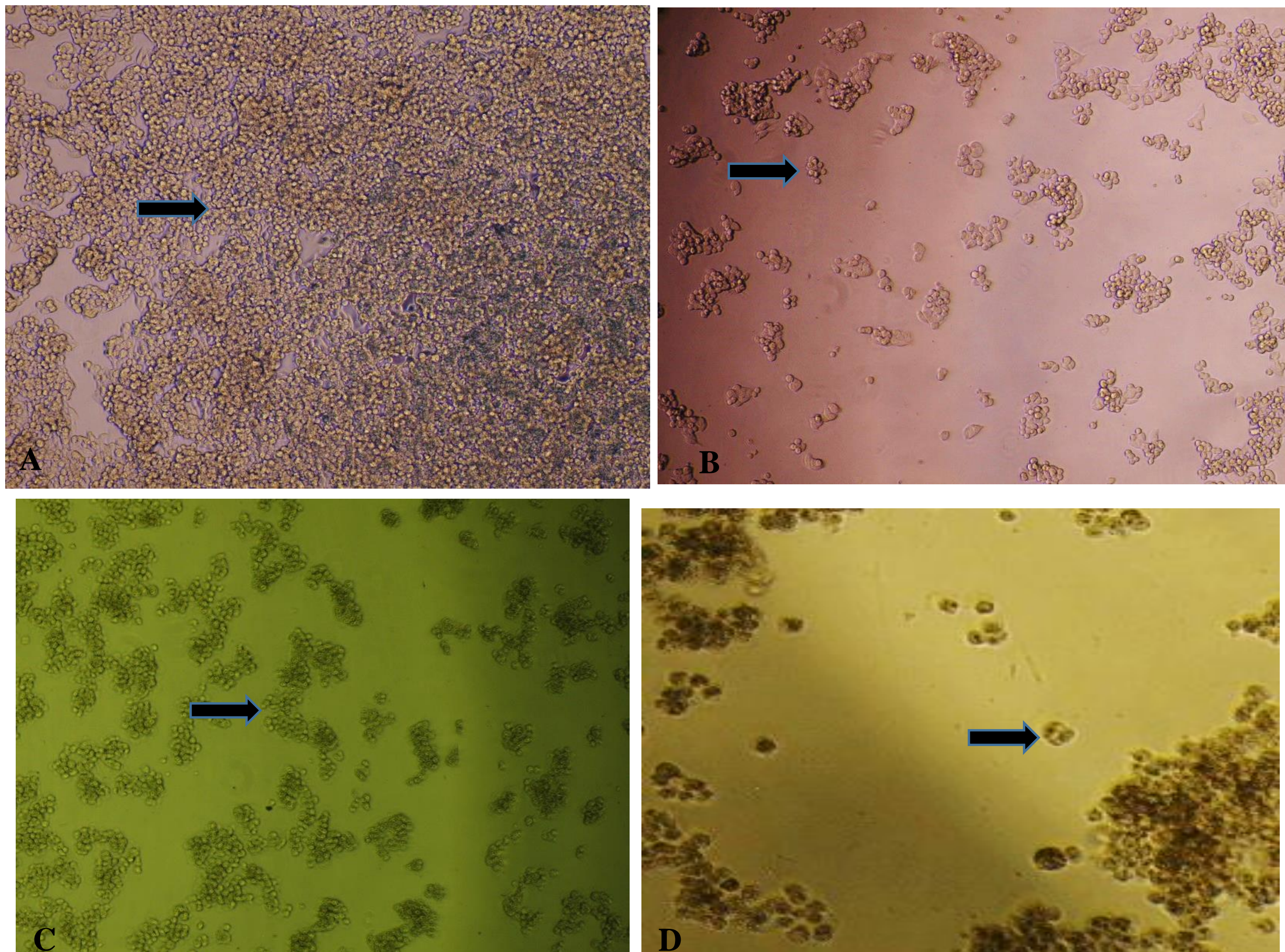

Figure No. 6
Morphological changes of Hep-G2 cancer cells. (A): of Hep-G2 cancer cells in the control group without treatment by hydroalcoholic extract of C. tinctoria (Negative control). (B): Positive control. (C): Morphological changes of Hep-G2 cancer cells after 48h treatment with hydroalcoholic extract of *C. tinctoria* at a concentration of 20 µg/mL, (D): Hep-G2 cancer cells after 72 h treatment with hydroalcoholic extract of *C. tinctoria* at a concentration of 20 µg/mL. Phase contrast analysis evidenced that the cells treated with the sample at different concentration showing apoptotic bodies, cell shrinkage and reduced in number and size

IC_{50} *values*

IC_{50} (inhibitory concentration) of hydroalcoholic extract on AGS cells after 24, 48 and 72 h was 1.187 µg/mL, 1.299 µg/mL and 1.152 µg/mL respectively (Table No. 1 & Figure No. 7). The hydroalcoholic extract demonstrated IC_{50} of 5.11 µg/mL, 3.902 µg/mL and 2.89 µg/mL on Hep-G2 cells after 24, 48 and 72 h respectively (Table No. 1 & Figure No. 8). No significant cytotoxic effect was detected on HUGU (Human Natural GUM fibroblast) cells, suggesting that the cytotoxic effect of hydroalcoholic extract was selective for cancer cells. (Figures No. 2-4).

Cancer cell line	IC_{50}		
	24 h	48 h	72 h
AGS	1.46 µg/mL	1.29 µg/mL	1.14 µg/mL
Hep-G2	5.15 µg/mL	3.92 µg/mL	2.89 µg/mL

Table No. 1
IC_{50} values of hydroalcoholic extract of *C. tinctoria* on AGS, Hep-G2 cancer cells after 24, 48 and 72 h

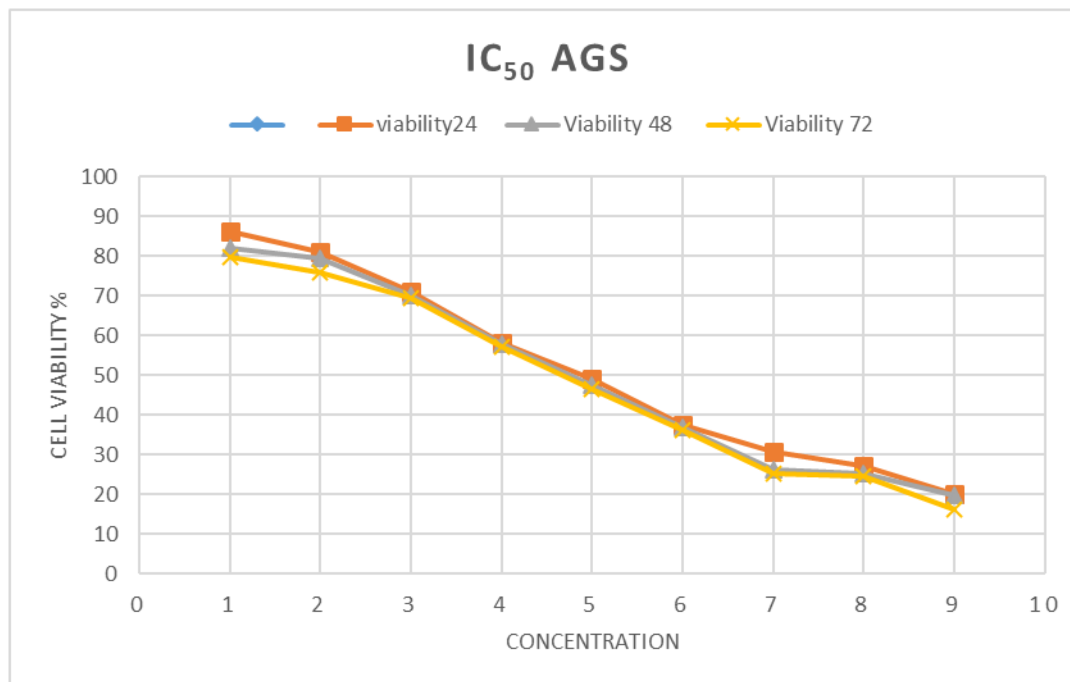

Figure No. 7
IC_{50} values of hydroalcoholic extract of *C. tinctoria* on AGS cancer cells after 24, 48 and 72h (Concentration µg/mL)

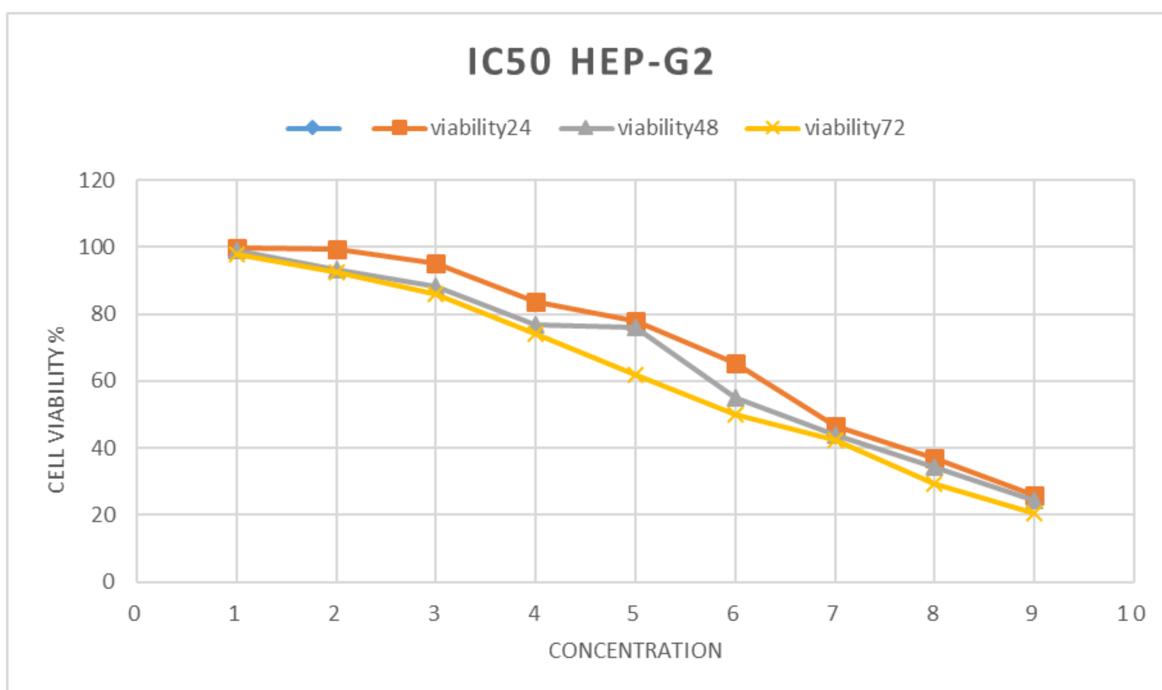

Figure No. 8
IC_{50} values of hydroalcoholic extract of *C. tinctoria* on Hep-G2 cancer cells after 24,48 and 72h (Concentration µg/mL)

DISCUSSION

Numerous edible plant-derived compound have been linked to the chemoprevention and treatment of cancer (Hsiao *et al.*, 2007; Tolomeo *et al.*, 2008; Zhang *et al.*, 2010; Jaiswal *et al.*, 2012; Mignet *et al.*, 2012; Ying *et al.*, 2012; Seguin *et al.*, 2013). Despite the extensive investigation carried out with flavonoids in the past decades, there are still quite a few parameters available, characterizing quantitatively the efficacy of polyphenolic compounds on certain cancer type. The requirement of anti-cancer drug candidates is that they should kill the proliferating cancer cells without affecting the normal cells (Shyam Kumar *et al.*, 2017).

In this way, the IC_{50} values of medical plants extracts measured using the cell derived from malignant tissues and determination of total phenolic and total flavonoid content are too scarce to reveal any certain specificity patterns.

In the present study, the cytotoxic effect of hydroalcoholic extract of *Cota tinctoria (Anthemis tinctoria)* on AGS and Hep-G2 cancer cells and natural fibroblast cells (HUGU Human GUM fibroblast cell) was examined. The result of this study showed that flower hydroalcoholic extract of *C. tinctoria* has significantly effective on AGS and Hep-G2 cancer cells growth at the different concentration and day and exhibited antiproliferative activity against AGS and Hep-G2 cancer cells and induced apoptotic cell death in AGS and Hep-G2 cancer cells while it does not have any significant toxic effect on natural fibroblast cells. Flavonoids have been demonstrated to suppress proliferation of various cancerous cells (Benavente-Garcia *et al.*, 2008). However, not all polyphenolic compounds share the same antiproliferative activity (Forni *et al.*, 2009) and depending on their structure, flavonoids display differences in the sensitivity and selective toward tumor cells (Rodriguez *et al.*, 2002; Nagao *et al.*, 2002; Morales & Haza, 2012). The sensitivities of cancer cells against flavonoids can be different depending on their derived tissues indicating that the cytotoxicity induced by flavonoids might be related to select cancer type (Kilani-Jaziri *et al.*, 2012). Even in the case of flavonoids with quite similar structures, there are compound-specific effects which are

relevant to modulate particular biochemical processes so that the development of certain neoplasms could be differentially influential pointing to the tissue-specific cytotoxic action (Zheng *et al*., 2002; Bonham *et al*., 2005). The effectiveness of flavonoids may vary also because of the different disease etiologies (Chen *et al*., 2000). Various species of the genus *Anthemis* have been used in the treatment of gastrointestinal disorders, stomachache, hemorrhoids, earaches, and deafness (Eser *et al*., 2017). Eser and colleagues also reported that all parts of the plans *Anthemis tinctoria* extracts exhibit better cytotoxic activity than the standard cancer drug 5-Florouracil.

cytotoxic effects of hydroalcoholic extract of *C. tinctoria* may be attributed to phenolic compounds such as a new flavonoid glycoside: tinctoside, which was isolated from the metanolic extract of the flowers of *Anthemis tictoria* L. (*Cota tinctoria*) for the first time (Masterova *et al*., 2005) and one new cyclitol glucoside: conduritol F-1-O-(6′-O-E-p-caffeoyl)-β-D-glucopyranoside, which has been isolated with four flavonoids nicotiflorin, isoquercitrin, rutin, and patulitrin and showed a strong scavenging effect in the DPPH radical assay. In addition, these isolated compounds also exhibited high inhibitory activity on soybean lipoxygenase (Papaioannou *et al*., 2007).

Orlando *et al*. (2019), measured the total phenol and flavonoid contents of EA, MeOH, and aqueous extracts of ATP and ACT of *Anthemis tinctoria* (*C. tinctoria*) and they reported that the phenolic content of ATP and ACT ranged from 26.46 to 100.09 mg GAE/g and 21.31 to 47.61 mg GAE/g, respectively. Highest phenolic content was observed in the MeOH extract and MeOH extract were rich in flavonoids. Whilst for ACT, MeOH extract contained the highest amount of phenols, followed by EA extract. Regarding the total flavonoid content, the results showed that EA (ATP: 45.82 an ACT: 46.26 mg RE/g) and MeOH (ATP: 48.54 and ACT: 45.08 mg RE/g) extracts of both species were rich in flavonoids. Based on their literature and comparison with standards, 15 flavonoid aglycones, twelve glycosides, and one caffeoyl-O-flavonoid were identified in the studied extracts.

Quercetin is one of the natural component from natural plant and induce cell apoptosis in many human cancer cell lines (Shang *et al*., 2018) which is reported in *C. tinctoria* (*A. tinctoria* L.) (Papaioannou *et al*., 2007). Shang *et al*. (2018), investigated the effect of quercetin on the apoptotic cell death and associated gene expression in human gastric cancer AGS cells and their result indicate that quercetin induced cell morphological changes and reduced total viability via apoptotic cell death in AGS cells.

Several studies have shown that quercetin controls cancer cell growth through the regulation of specific signaling pathways, such as decreasing oncogene expression, inducing malignant cell apoptosis and inhibiting angiogenesis (Yuan *et al*., 2012; Pratheeshkumar *et al*., 2012) Zhou and colleagues in 2017 showed that quercetin can significantly inhibited the proliferation of HepG2 cells and inducing apoptosis, possibly through the participation of cyclin D1 regulation. In another study Zhao *et al*. (2014), was found that quercetin could induce apoptosis in human liver cancer HepG2 cells with overexpression of FANS. This apoptosis was accompanied by the reduction of intracellular FANS activity and could be rescued by 25 or 50 μM exogenous palmitic acid, the final product of FASN-catalyzed synthesis. These results suggested that the apoptosis induced by quercetin was via the inhibition of FANS. These findings suggested that quercetin may be useful for preventing human liver cancer.

Rutin is one of the flavonoids that reported in *C. tinctoria* (*A. tinctoria* L.) (Papaioannou *et al*., 2007) with many biological activities including antiallergic, anti-inflammatory, and anti-prolifretive and anti-carcinogenic properties (Shyam Kumar *et al*.,2017) Rutin exert its anticancer effect by inducing apoptosis by arresting the G0/G1 phase in the cell cycle. This cell cycle arrest can be attributed by acting upon Bcl-2 family protein which regulates all major types of cell death. Shyam kumar *et al*. (2017), showed that rutin along with Ascorbic acid (Vitamin c) and (Vitami K) shows cytotoxic activity on Hep-G2 cancer cell line. Also Sghaier *et al*. (2016), repored that rutin inhibits proliferation, attenuates superoxide production and decrease adhesion and migration of human cancerous cells and the viability of cancerous cell was inhibited by rutin.

Patuletin was isolated from the metanolic extract of of the flowers of *Anthemis tinctoria* L. (*C. tinctoria*) (Masterova *et al* 2005). Report results clearly show that quercetin, quercetagetin and patuletin all have good cytotoxic activity against different cancer cell lines, and that they achieve cell elimination through the ordered cell-death process called apoptosis. In addition, their results indicate that these compounds induce the intrinsic apoptotic pathway. In addition to demonstrating the pro-apoptotic action exerted by quercetin, quercetagetin and patuletin inside tumor cells, we were also able to determine that these compounds show a selective

effect on non-tumor cells, since when normal lymphocytes were treated with the doses applied to the cancer cell lines After treatment with quercetin, quercetagetin or patuletin, the morphology of the cells was clearly altered, as they showed shrinkage, evidenced by the loss of the polyhedral form. Compact nuclei and apoptotic bodies were also visible (Alvarado-Sansininea *et al.,* 2018).

Quercetin and rutin and apigenin are known major flavonoids in Asteraceae family (Dagnon *et al*., 2013), and have been shown to strongly inhibit the growth of cells and trigger apoptosis of human cancer cells (Shyam Kumar *et al*., 2017; Zhou *et al*., 2017).

Studies show a significant difference at three treatment times (24, 48 and 72 h) the higher the concentration and duration of the effect of the hydroalcoholic extract, the greater its cytotoxic effect on AGS and Hep-G2 cancer cells. However, findings indicate that hydroalcoholic extract of *C. tinctoria* was highly cytotoxic on AGS cancer cells than on Hep-G2 cancer cells, while it does not have severe toxicity on HUGU Natural Human Gum fibroblast cells (Table No. 1). HUGU cells were significantly less sensitive to hydroalcoholic extract of *C. tinctoria*.

Also Bardaweel *et al*. (2014), and colleagues investigated on Antioxidant, antimicrobial and antiproliferative activities of *Anthemis palestina* essential oil and the results obtained indicate potent cytotoxic activities, especially against Hela cell line, BJAB cell line and Caco-2 cell line and high potential of *Anthemis palestina* essential oil as bioactive oil, for nutraceutical and medical applications, possessing Antioxidant, antimicrobial and antiproliferative activities (Bardaweel *et al*., 2014).

CONCLUSION

These results suggest that by increasing incubation time, the extract showed better cytotoxic activity. Moreover, the extract demonstrated more cytotoxic activity on AGS cells compared to Hep-G2 cells. In conclusion, *C. tinctoria* seems to have a good potential to be considered as a source for anticancer drug discovery fermentation of *C. tinctoria* could be applied to develop natural antioxidant and anticancer products. The result is an early step in examining and identifying anticancer compounds. Studies have already shown that plant compounds and their derivatives can be an effective weapon and be considered as part of the standard protocols for cancer treatment and prevention. Due to the wide variety of plants that exist, researchers face a long path of continued research in this field.

ACKNOWLEDGEMENTS

The authors acknowledge the insightful comments from Dr Majd's reviewers who have meticulously read the research report and provided invaluable advice to improve the quality of the report, and also thank the Islamic Azad University laboratory.

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