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FIRST REPORT OF THE ANTICANCER EFFICIENCY OF AGARICUS BRASILIENSIS MUSHROOM ON HUMAN EMBRYONIC LIVER WRL68 AND HUMAN PANCREATIC ASPC-1 CANCER CELLS THERAPY

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Abstract

Mushrooms are food traditionally consumed in Asia, Europe and America. They are being studied for medicinal benefits. Extensive studies have shown that Agaricus brasiliensis mushroom used as a medical productto combat cancers.

Our data reveal that the determined inhibitory concentration fifty(IC50) values were observed maximum dose responses (IC50) of WRL68 andAsPC-1 cancer cells reported of 172.6 µg/ml and 158.2 µg/ml respectively at 2.23 and 2.1 µg/ml ethanolic mushroom concentrations. The highly cytotoxic activity of the extract on growth inhibition AsPC-1 and WRL68were generally observed 97.9% and 95% at extract concentrations of 25 µg/ml and 50µg/ml respectively.

Finally, Phytochemical profile of Agaricus brasiliensis mushroom extract found to beflavonoids, glycosides, saponins, phenols,alkaloids, tannins.The extracts of Agaricus brasiliensiswas tested throughGas Chromatography-Mass(GC-MS). There were five different compounds analyzed from the extracts of Agaricus brasiliensis. The compounds in the ethanolic extract of A. brasiliensis mushroom were comprised mostly of Acetic acid ethyl ester(38.39%),followed by(3-Methyl-oxiran-2-yl)-methanol (34.71%), Chlorbromuron (22.86%), n-Hexadecanoic acid (3.85%) and Heptane, 1-(1-butenyloxy) (0.19%).

However, no studies were done using Agaricus brasiliensismushroom extract against WRL68 and AsPC-1 human cell lines. Therefore, the anticancer efficiency of Agaricus brasiliensis mushroom on human embryonic liver WRL68 andhuman pancreatic AsPC-1 cancer cellstherapy is being reported for the first time in the current study.

Keywords: Agaricus brasiliensis, Phytochemical profile, MTT assay,GC-Mass, WRL68, AsPC-1.

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1. Introduction

According to the American Cancer Society, WRL68 and AsPC-1 are a particular name for a cancer that starts with human's liver and pancreas respectively. The latest estimates of the liver and pancreatic cancers deaths in the world are accounting for more than 700000 and 330000 deathsrespectively(American Cancer Society, 2021).

Current therapy involves a mixture of radiation, surgery and chemotherapy, although the level of relapse seems to be extremely high, Therefore, the lack of clinical strategies and the desire to support the patient's health demands the assessment of an additionalor supplementary therapeutic approach(Buchanan et al., 2005; Nakamura et al., 2019). In the recent decades, the scientific experiment and technological advances of novel drugs have expanded and tumoricidal substances have become increasingly important(Patridge et al., 2016).

Currently,Mushrooms were used as humans' nutritionfor thousands of years and are remarkablypoor in calories but rich in minerals, vitamins, fibers, and essential amino acids(Lima et al., 2011; Tepsongkroh et al., 2020). Among of these mushrooms, Agaricus brasiliensis mushroom, an edible fungus in the agaricaceae family. It was historicallyused as a medical food to combat cancer, diabetes, hyperlipiodemia, coronary artery disease, and chronic liver disease and is widely believed to activate the immune response(Misgiati et al., 2021). They considered as nutritional valuedue to their diverse chemical properties and large ratio of phytochemical components, providingnumerous health benefits to those who eatthem, in addition to theirlack of toxicity (Orsine et al., 2012). The majority of mushroom content constitutes water (90%), protein (2-40%), carbohydrate (1-55%), fiber (3-32%), and ash (8-10%)(Zivkovic et al., 2017).

To our knowledge, this is the first discovery in the world on the cytotoxic activities of Agaricus brasiliensis extract against WRL68 and AsPC-1 human cell lines. Based on the above findings, the goal of this work was to discover the anticancer efficiency of Agaricus brasiliensis mushroom on human embryonic liver WRL68 and human pancreatic AsPC-1 cancer cells therapy.

Materials and Methods

Preparation of Agaricus brasiliensismushroom extracts

The Agaricus brasiliensismushroom was collected from a local producer in Al- Anbar Governorate in Iraq, cleaned, dried at 40˚C, grinded and then weighed. The mushroom dried powder20g was used for 200ml of solvent 70% ethanol, and then extracted by using soxhlet. After 24h, The solution was centrifuged for 15min at 1000 rpm /min, and then collected liquid phase was used for further process. The liquid portion was dried at 50 ˚C in a rotary evaporator and then preserved at -20 ˚C for further studies.

In-vitro Anticancer activity

The anticancer efficiency of ethanolic extract from Agaricus brasiliensismushroom against WRL68 and AsPC1 human cell lines that obtained from the Iraqi Center For Cancer Research in Al- Mustansiriyah university. Cells have been grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin at 37˚C in a humid condition of 5% CO2 for 24h.

Cell viability MTT assay was includedby the standard instructions(Promega Corporation, Madison, WI, USA).At first, the 96-well tissue culture platewas filled with 100 µl/well of cells(106cell/ml). Various concentrations of Agaricus brasiliensis mushroom extract test solution were prepared toevaluate cytotoxic effect against two examined cell line(400, 200, 100, 50, 25, 12.5, 6.25 µg/ml) in water.

After that, 100µl of different concentrations was applied to each well in an incubator at 37˚C with 5% CO2humid conditions for 24h. Duringthe incubation, 10µl of 5mg/mlMTT solution was transferred to each well and incubation at 37˚C for 2h.

An extraction buffer(20% sodium dodecyl sulphate(SDS) and50% dimethylformamide) introduced to each well and incubated at 37˚C overnight.Thenegative control wells included cells with the medium in 0.4% DMSO.

The cell suspension absorbance was calculatedusing ELISA Microplate Reader(Bio Tek, USA) at 570nm.

Cell viability was described as the ratio of the mean absorbance of the treated cells to that of control cells. The tumors cells' sensitivity to the extract was represented as IC50 values.

This experiment was replicated three times, and the statistical data was analysed to give the final results.

Phytochemical profile analysis

phytochemical profile was performed by standard instructions (Trease and Evans, 1989; Harborne, 1998).

Gas Chromatography-Mass Spectrometry

By using a high-temperature column, Agilent Technologies (SHIMADZU-Japan) bought a high-temperature column (Inert cap IMS; 30 m x 0.25 mm id x0.25um film thickness) for GC-MS research. Each sample's derivatization was withdrawn. The injector and detector were calibrated to 280°C, wherease the original column heated at 100°C. The column was loaded with a 5 µl sample volume and operated in split (1:10) system. The sample was heated to 225°C at a level of 12.5°C/min after 1 minute. After that, this sample was gradually increased to 300°C at a level of 7.5°C/min. The helium carrier gas optimized to preserve a stable flow rate of 17.5mL/min. The data reported and analyzed through Agilent GC-Mass Solution (SHIMADZU-Japan) and postrun software. The compounds were detected by matching their mass and NIST database.

Data analysis

For statistical research, the program SPSS 20.0 has been used. Three different values have been represented as mean + SD values. In all instances, the adopted levels was 5% ($p < 0.05$).

Results and Discussion

Cell line growth and cytotoxicity assay

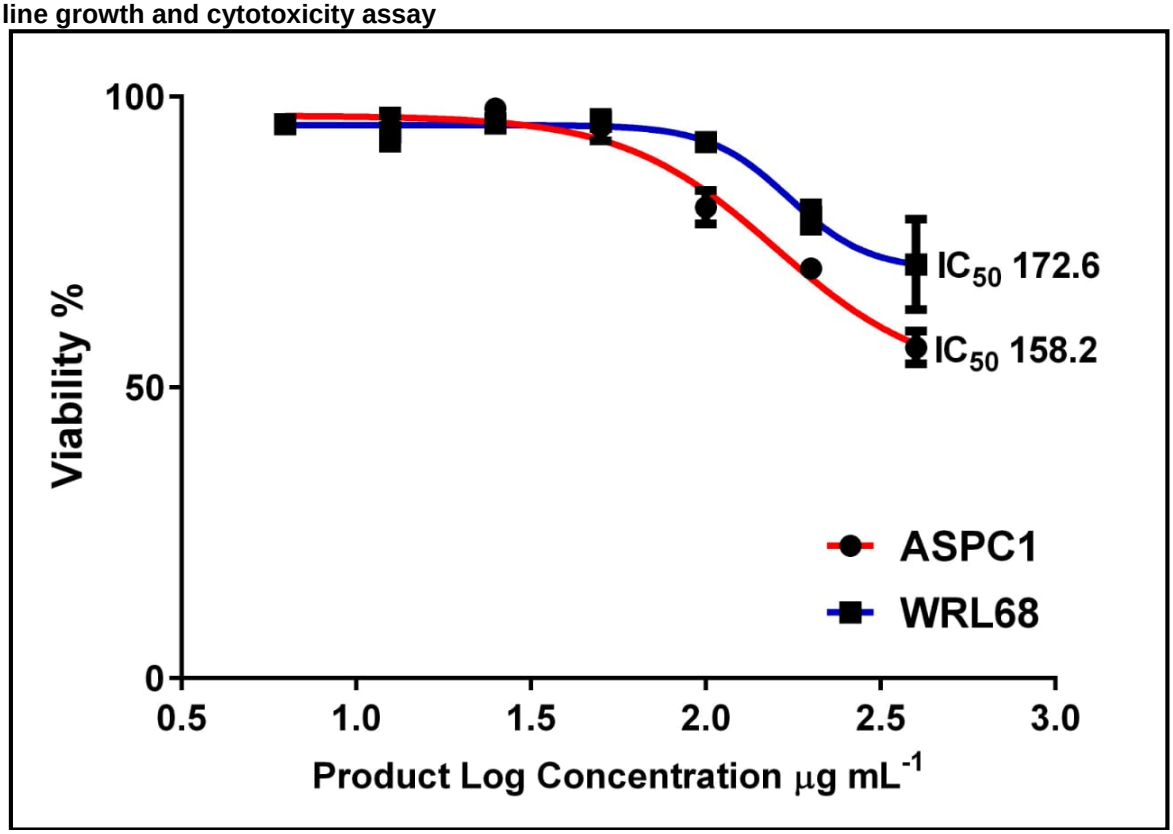
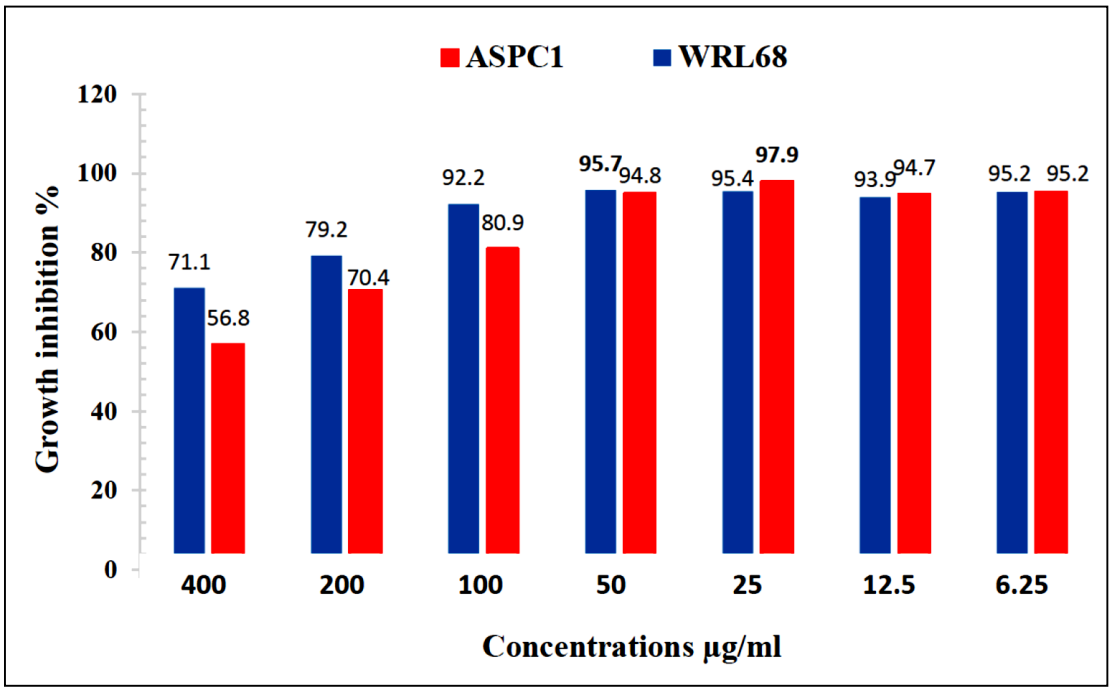


Figure1:Dose responses (IC50) of anti-tumor mushroom on WRL68 and AsPC-1 human cell lines. Cells have beengrown in Dulbecco's Modified Eagle medium supplemented with 10% fetal bovine serum and subjected to ethanolic extract of Agaricus brasiliensis mushroom at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0µg/ml for 24h. In each experiment was replicated three times and two different experiments were conducted. Mean ±SD(n=3) are values shown.

In our experiments,the cytotoxic of the ethanolicextract A. brasiliensismushroom on WRL68 and AsPC-1human cell lines were shown to be a dose-responsive manner which found significant variation compared to that control (Fig 1).These results indicated that ethanolic extract A. brasiliensis mushroom significantly and specifically inhibited the proliferation of human embroyonic liver WRL68 and pancreatic AsPC-1 cell lines relative to control.

Our data have shown that the maximumdose responses(IC50) ofWRL68 and AsPC-1reported of 172.6 µg/ml and 158.2 µg/ml respectively at 2.23 and 2.1 µg/ml ethanolic mushroom concentrations compared to control. Although this is the first work on the effect of ethanolic extract A. brasiliensis mushroom was recorded to inhibit the viability of human embroyonic liver WRL68 and pancreatic AsPC-1 cell lines. Simply, although it was not related to A. brasiliensis, another mushroom extract from Cyathusstriatushas been discovered that growth inhibition againstHPAF-II and PL45 human pancreatic adenocarcinoma cells can be reached even insmall doses and short exposure time (Sharvitet al., 2012). Furthermore, the MMH01 compound obtained from Antrodiacinnamomea mushroom was successful in inhibition toward BXPC3 pancreatic cancer cells

(Chen et al., 2009). Another work reported in(Yu et al., 2012) registered as anti-proliferative effectstoward PANC-1 and AsPC-1 pancreatic cancer cellsbyantroquinonol derivative collected from Antrodiacamphorate mushroom. Additionally, Cheng et al., (2013) demonstrated that triterpenes extracted from Poriacocos acted as anti-proliferative agents against four pancreatic PANC-1. MIAPaCa-2, AsPc-1 and BxPC-3 carcinoma cell. Also, Ghosh and Sanyal, (2020) observedthe ethanolic extract of Calocybeindica mushroom inhibited proliferation of AsPC-1 pancreatic cell line.



Figure(2): The effect of various concentrations of A. brasiliensis mushroom ethanolic extract on growth inhibition of two human embryonic liver WRL68 and human pancreatic AsPC-1 cancer cells after 24hr incubation time.

In figure(2), Cytotoxicity was determined at a high rate of growth inhibition97.9% and 95% in AsPC-1andWRL68 at of 25 µg/ml and 50µg/ml concentrations respectively as compared to the cell control.
In data study, A. brasiliensis mushroom extract detected that improving anticancer activities through existenceseveral of the bioactive compounds.
Antitumor activity of mushroom derivatives on AsPC-1 and WRL68 cell lines. Till now, no study has been performed about the anticancer effect of A. brasiliensis mushroom on AsPC-1 and WRL68 cell lines, however, there are a few experimental data supporting potential anti-tumor activity of this mushroom against another human cell lines.
Ziliotto., (2014)was observed antitumor activity of dichloromethane/ methanol and hexanic A. brasiliensisextracts mushroom at 250µg/ml for K-562, NCI-ADR, NCI-460, UACC62, OVCAR, HT-29 and 786-0 human cancer lines. Similarly, Shimizu et al., (2016)explained the cytotoxic effects of Agarol from A. brasiliensis mushroom against human carcinoma cell lines A549, MKN45, HSC-3 and HSC-4.Another study cytotoxic effect of A. brasiliensisextracts on human myeloma cells and leukemic cells(Tangent et al., 2017).Other study has shown that the hot water extraction of A. brasiliensismushroom observed inhibition toward human leukemia Jurkat cell(Kozarski et al., 2014).

Phytochemical profile analysis
Phytochemical profile of the ethanolic extract of Agaricusbrasiliensisachieved the occurrence of flavonoids, glycosides, phenols,saponins,alkaloids, tannins as shown in table(1), the mean of pH extracts was (5.5-6).
Table(1): phytochemical profile of the ethanolic extract of A.brasiliensis.

Effective compounds	A. brasiliensis mushroom extract
Flavonoids	+
Glycosides	+
Saponins	+

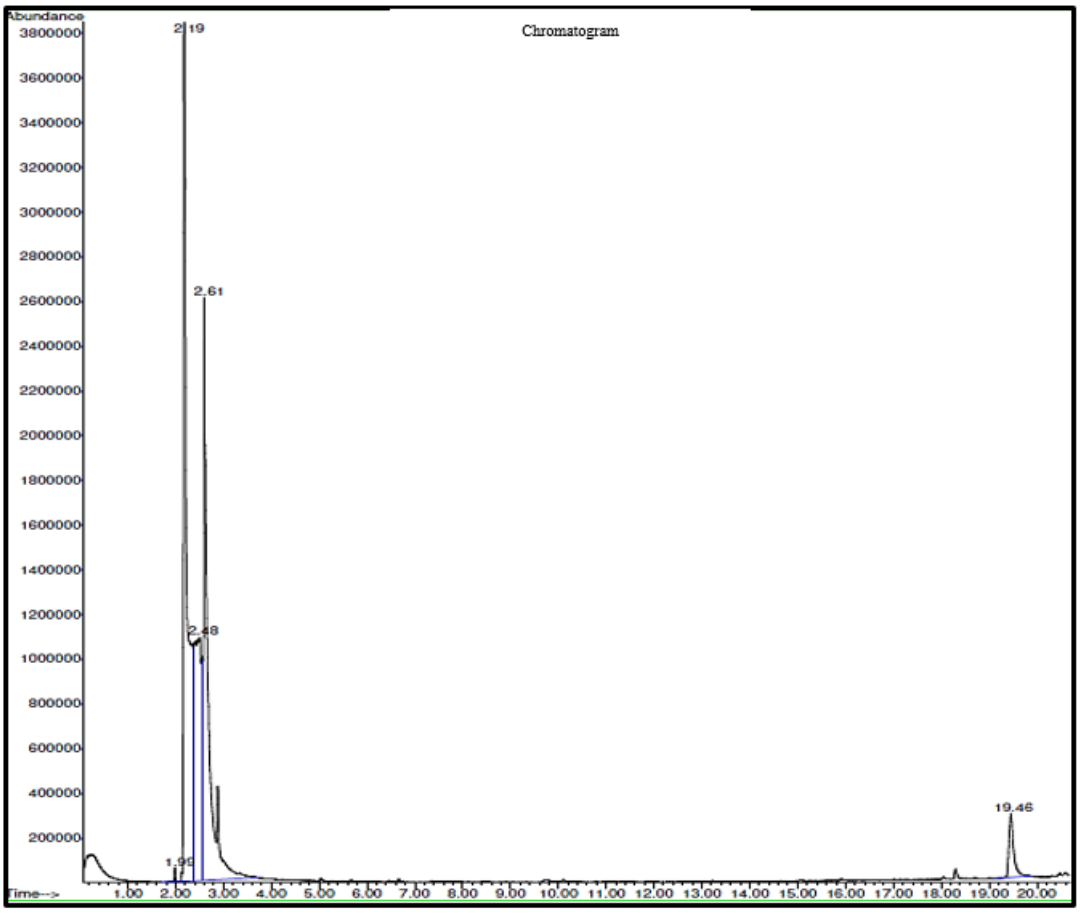
Phenols	+
Alkaloids	+
Tannins	+

Several studies on many substances of A. brasiliensis such as sterol,sesquiterpenes, riboglucan,anthraquinones, glucomannan, sodium pyroglutamate, derivatives of benzoic acid, lectins, RNA-protein complex, organic acids, amino acids, lactic and fumaric acid, steroids, quinolones, phenolic compounds and polysaccharides such as β-glucans have been reported as potential bioactive substances (Motoi, 2012; Da Silva et al., 2017; Moukhaet al., 2020; Venkateshgobiet al., 2021).
polysaccharides are the most well-known and have many tumor preventative constituents and immunomodulatingeffects. A. brasiliensis polysaccharide comprises of 57.7% glucose, 27.7% galactose, 7.3% mannose/ xylose, and 4% fucose(Martinset al., 2017). Among of these polysaccharides, β-glucanrefers to a glucose moleculefound in medical mushrooms. It showsimmunomodulatory, antitumor,antiproliferativepropertiesin cancer patients by activation of immune response(Cardozo et al., 2014). Moreover, Antihyperglycemic, antihypertriglyceridemic, antihypercholesterolemic, and antiarteriosclerotic properties have been observed in β-glucans from A. brasiliensis(Wei et al., 2020).
Agaricusbrasiliensis is abundant incertain natural compounds including polyphenols and polysaccharides which have antioxidant, antitumor, and immunomodulation(Hetlandet al., 2020).
Lectins can be seen to be pharmaceutical sources with tumorcidal activities in animals and human trials. It causes cytotoxicity and apoptosis through attaching to cancer cell membranes which suppress tumor growth(Sendraet al., 2010).
Ergosterolis the vitamin D biosynthesis in the lipid fraction of Agaricales extracts. It affectson human cancer cells through antitumor, antiproliferation, and antimigratory effects and inhibitsangiogenesis(Shimizu et al., 2016).
Arginine was prescribed to cancer patients as a nutritional aid. It has been linked to a decrease in tumor growth and metastasis progression. Hence it has positive impacts to the immune response, weight gain, and cancer patient recovery period. (Tada et al., 2011).
Another report ofblazeispirol A, collected from A. brasiliensis, reduces growth inhibition ofHep 3B hepatomacells (Wang et al., 2013).
Theagaritinecomponentwas found in A. brasiliensis.It has tumorcidalproperties in leukemic cells (Nagaoka et al., 2006).
The Blazeinderivative occurs in A. brasiliensiswhich considered as an anti-proliferative effecttowards LU99 human lung cancer cells (Itoh et al., 2008).

Evaluation of Gas Chromatography-mass Spectrometry for Fungal Extracts
As shown in the figure3, GC- MS of the chromatogramethanolic extract of A. brasiliensis showed the presence of about five peaks. Their name, molecular formula, molecular weight, retention time (RT) andpeaks area(%)are listed in table(2).
The compounds in the ethanolic extract of A. brasiliensis mushroom were comprised mostly of Acetic acid ethyl ester (38.39%), followed by (3-Methyl-oxiran-2-yl)-methanol (34.71%), Chlorbromuron(22.86%), n-Hexadecanoic acid (3.85%) and Heptane, 1-(1-butenyloxy) (0.19%).
However, many of these identified constituents have been found to possess several pharmacological activities i.e. antitumor, antimicrobial, antioxidant, antiviral, anti-arthritis, antifungal, insecticidal and other therapeutic potentials(Mohamed, 2012; Gyorfiet al., 2013; Alshammaet al., 2017; Elkhateebet al., 2019; Adeoye-Isijolaet al., 2021).

Table(2): Major phytochemicals obtained in the ethanolicextract ofA. brasiliensis.

No. of peak	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (RT)	peaks area (%)
1	Heptane, 1-(1-butenyloxy)	C11H22O	170.29	1.99	0.19
2	Acetic acid ethyl ester	C4H8O2	88.1051	2.19	38.39
3	Chlorbromuron	C9H10BrClN2O2	293.545	2.48	22.86
4	(3-Methyl-oxiran-2-yl)-methanol	C4H8O2	88.11	2.61	34.71
5	n-Hexadecanoic acid	C16H32O2	256.4241	19.46	3.85
Total identified					100



Figure(3): GC-MS Chromatogram of the ethanolicextract of A. brasiliensis.

Conclusions

This is first report in world onanethanolic extract of mushroom A. brasiliensiswhich is effective in inhibiting cell growth of WRL68 and AsPC-1 cell lines, indicating that it may be used as an alternative therapy in the treatment of liver WRL68 and pancreatic AsPC-1 cell lines. Thus, it is suggestedthat the anticancer efficacy of the GC-MS detected compounds be evaluated in order to discover new therapeutic approaches for cancer treatment in future. Further studies are required to reveal the important chemical constituents responsible for anti-proliferative activity.

Conflict of interest

The author declares no conflict of interest.

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