Archives of Razi Institute, Vol. 77, No. 2 (2022) 753-760

DOI: 10.22092/ARI.2021.356860.1931

Original Article

Pharmacognostic Profile and Screening of Anti-Proliferative Potential of Methanolic Extract of *Tripterygium wilfordii* Plant on WRL-68 Cell Line and Function of Polycystin-1

Hamdan, N. T[1 *], Abdalkareem Jasim, S[2], Khayoon Abed Al-Abboodi, A[3]

1. Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
2. Department of Medical Laboratory Techniques, Al-Maarif University College, Anbar, Iraq
3. Ministry of Education, Iraq

Received 12 November 2021; Accepted 2 December 2021
Corresponding Author: noor.t.hamdan@uomustansiriyah.edu.iq

Abstract

Tripterygium wilfordii is a medicinal plant that plays a crucial role in health care programs, especially in developing countries, and had anti-tumor, anti-inflammatory, anti-fertility, anti-bacterial, and other therapeutic effects. This study was designed to determine the anti-proliferative effects of methanolic extract of *T. wilfordii* on the WRL-68 cell line and the function of polycystin-1 (PC-1). The half-maximal inhibitory concentration (IC_{50}) values were recorded in WRL-68 and AsPC-1 cell lines as 193 µg/ml and 149.2 µg/ml, respectively, at 2-2.55 and 2-2.2 µg/ml methanolic plant concentrations. The maximum cytotoxic activities of the extract on the growth inhibition of WRL-68 and AsPC-1 were generally observed at 97.64% and 95.94% at extract concentrations of 50 µg/ml and 25 µg/ml, respectively. The pharmacognostic profile of *T. wilfordii* extract was found to be alkaloids, tannins, terpenoides, flavonoids, glycosides, and phenols. The extracts of *T. wilfordii* were tested through gas chromatography-mass spectrometry showing four peaks representing mostly of 3-Oxobutanol; ethyl acetate; acetic acid ethyl ester; chlorbromuron; 1-(methylthio)-, (E)-; n-Hexadecanoic acid; tetradecanoic acid; and 9-Octadecenoic acid. Therefore, the results of this study revealed that the methanolic extract of *T. wilfordii* was more potential in inducing anti-proliferative activity of WRL-68 and AsPC-1 human cell lines than the control. In addition, the current study was the first study that reported the anti-proliferative potential of *T. wilfordii* in the treatment of human embryonic liver WRL-68 cancer cells.

Keywords: Anti-proliferative, GC-MS, MTT, Pharmacognostic profile, *Tripterygium wilfordii* plant

1. Introduction

According to the American Cancer Society (1), WRL-68 and AsPC-1 are particular names for hepatic human fetal cell line and human pancreatic cancer, respectively. The latest mortality rates due to the liver and pancreatic cancers in the world have been estimated at more than 700,000 and 330,000 deaths, respectively (1).

Polycystin-1 (PC1) is well known for its crucial role in autosomal dominant polycystic kidney disease where a mutation in the encoding gene results in the generation of fluid-filled renal cysts as well as cysts in other epithelial organs, including the liver and the pancreas. The PC1 function has been explored in the context of polycystic kidney disease where a mutation in the PC1 gives rise to a complex cell phenotype, characterized by increased cell proliferation and apoptosis, de-differentiation, disturbed planar cell polarity, extracellular matrix alterations, and abnormal fluid secretion.

Nowadays, conventional treatment approaches for cancer patients, such as chemotherapy and surgery,

have not been demonstrated to be completely effective for numerous cancers (2). Therefore, new medications from traditional medicine are being pursued to avoid this global warming-related disease. According to the Food and Drug Administration, 74% of traditional medicine is used in cancer treatment (3). Among these, medicinal plants with various biological effects are utilized as a source of traditional medicines, especially in anti-tumor properties, because of having fewer side effects and being cost-effective, protective, and effectively prognostic (4, 5).

Tripterygium wilfordii is a medicinal plant belonging to the Celastraceae family, is cultivated exceedingly in East Asian countries, and has been utilized in conventional medicine for the treatment of swelling, fever, chills, sores, and joint pain and inflammation (6, 7). Additionally, it has been employed to combat cancers, rheumatoid arthritis, chronic nephritis, hepatitis, systemic lupus erythematosus, infertility, Crohn's disease, ankylosing spondylitis, and a number of skin diseases (8-10).

Based on the above aspects and detailed past previous works, *T. wilfordii* was chosen in this study as a model plant to discover its therapeutic effects on WRL-68 and AsPC-1 cancer cell lines proliferation.

2. Materials and Methods

2.1. Preparation of *Tripterygium wilfordii* Methanolic Extracts

Fresh leaves of *T. wilfordii* were gathered from a botanical garden of Al-Mustansiriyah University, Baghdad, Iraq, in September 2020, and identified by the members of the Department of Biology, Al-Mustansiriyah University. The leaves were cleaned and cut into small pieces. They were then properly dried at 40°C, ground, and weighed. The dried powder of *T. wilfordii* leaves (50 g) was used for 600 ml of 70% ethanol solution, and subsequently extracted using a Soxhlet extractor. After 24 h, the solution was centrifuged for 15 min at 1,000 rpm/min, and the collected liquid phase was used for further process. The liquid portion was concentrated at 50°C in a rotary evaporator and then dried in a lyophilizer to completely remove the solvent and obtain the dry methanolic extract. Finally, the dry methanolic extract was stored at -20°C for further studies.

2.2. Cell Culture

WRL-68 cells (ATCC, USA) were maintained in Eagle's Minimum Essential Media (Flowlab, Australia) containing 10% fetal calf serum (PAA, Austria) and 100 µg/ml penicillin/streptomycin (Flowlab, Australia) at 37ºC in an atmosphere containing 5% CO_2.

2.3. Detection of Anti-Proliferation Potential by MTT Assay

The anti-proliferation potential assay was performed by methanolic extract from *T. wilfordii* against WRL-68 and AsPC-1 human cell lines that we obtained from the Iraqi Center for Cancer Research in Al-Mustansiriyah University. Different concentrations from the *T. wilfordii* methanolic crude extract (6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml) were prepared in Dulbecco's Modified Eagle Medium including 2% fetal bovine serum and 2% antibiotics at 37°C in a humid condition of 5% CO_2 for 24 h. The anti-proliferative effects of the studied extract were investigated on tested lines using the colorimetric 3 (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (11). Briefly, 100 µl of cell lines (10^6 cells/well) were injected into 96-well microtiter plates. After being in a 5%-CO_2 incubator at 37°C for 24 h, the cells lines were loaded with 100 µl of different extract concentrations. The negative control wells contained cells with the medium in 0.4% dimethyl sulfoxide (DMSO). The treated or nontreated cells were incubated for 2 days at 37°C in a 5%-CO_2 incubator. The culture medium was then removed and substituted with 100 µl of MTT solution (5 mg/ml) for 4 h at 37°C in a CO_2 incubator. MTT solution was then scrapped and changed through 50 µl DMSO/well. After 30 min at 37°C, the optical densities were used through an enzyme-linked immunosorbent assay reader at 540 nm.

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

represented as half-maximal inhibitory concentration (IC_{50}) values. This experiment was replicated three times, and the statistical data were analyzed to give the final results.

2.4. Pharmacognostic Profile Analysis

A pharmacognostic profile was performed by standard instructions (12, 13).

2.5. Gas Chromatography-Mass Spectrometry Analysis

The derivatization of each sample was withdrawn. The injector and detector were calibrated at 280°C, whereas the original column was heated at 100°C. The column was loaded with 5 µl of sample and operated in a split (1:10) system. The sample was heated to 225°C at a level of 12.5°C/min after 1 min. Afterward, this sample was gradually increased to 300°C at a level of 7.5°C/min. The helium carrier gas was optimized to preserve a stable flow rate of 17.5 ml/min. The compounds were detected by comparing their relative retention time and mass spectrum data of the specific compound with those of the common prominent compound available in the library of the National Institute of Standards and Technology.

2.6. Data Analysis

The collected data were analyzed in SPSS 20.0 software using one-way ANOVA. Three different values were represented as mean ± SD values. In all instances, the *P*-value of < 0.05 was considered significant.

3. Results and Discussion

3.1. Anti-Proliferative Estimation of *Tripterygium wilfordii* Methanolic Extract

Initially, an MTT assay was performed against WRL-68 and AsPC-1 cell lines to investigate the anti-proliferative property of methanolic extract of *T. wilfordii* leaves. It was observed that after treating the cells with 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml of methanolic extract for 24 h, methanolic plant extract showed to be more potent in anti-proliferative activity than the control in both tested cell lines (Figure 1). The *T. wilfordii* methanolic extract showed a dose- and time-dependent induction of anti-proliferative activity in these tested cell lines. The IC_{50} values were recorded in WRL-68 and AsPC-1 cell lines as 193 µg/ml and 149.2 µg/ml, respectively, at 2-2.55 and 2-2.2 µg/ml methanolic plant concentrations in a 24-hour treatment. Hence, it was concluded that the anti-proliferative effects of *T. wilfordii* methanolic extract were specific to cancer cells.

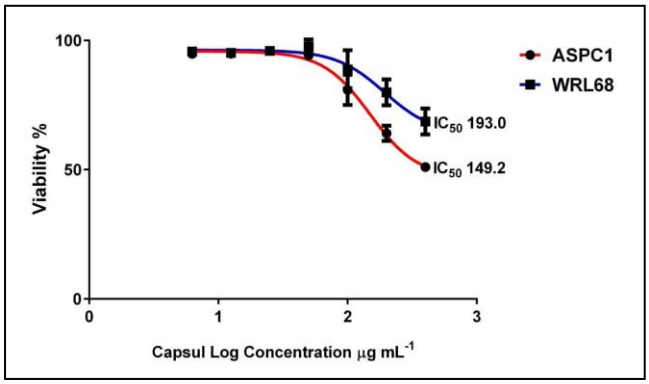

Figure 1. Dose responses (IC_{50}) of the anti-proliferative potential of *Tripterygium wilfordii* plant on WRL-68 and AsPC-1 human cell lines

Cells were seeded in Dulbecco's Modified Eagle medium and subjected to methanolic extract of *T. wilfordii* at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0µg/ml for 24 h. Each experiment was replicated three times, and two different experiments were conducted. The values are shown as mean ± SD (n=3) at the *P*-value of < 0.05.

In the current study, it was first observed that *T. wilfordii* extract inhibited the proliferation of embryonic liver WRL-68 cell lines. Although not related to *T. wilfordii*, another medicinal plant extract from *Astilbe rivularis* has been discovered to have anti-proliferative activity on human WRL-86 and HEK-293 cell lines (18). Moreover, Rawa'a, Hassawi (19) investigated the effect of *Taraxacum officinale* extract on human WRL-68 and MCF-7 cell lines.

In table 1, the growth inhibition induced by the methanolic extract of the *T. wilfordii* leaves is reported to be statistically significant ($P<0.05$). The

maximum growth inhibitions were reported at 97.64% and 95.94% in the WRL-68 cell line and AsPC-1 cell line, respectively, compared to the cell control. Therefore, it was evident that the methanolic extract of *T. wilfordii* leaves was a good repository of anti-proliferative property-rich molecules. This result was in agreement with those of some studies showing the anti-cancerous effect of *T. wilfordii* on the pancreatic cell lines. Wang, Matta (20) found that *T. wilfordii* potently declined cell growth toward Hela and PANC-1 cell lines. Moreover, statistics have shown that triptolide limits the growth inhibition of pancreatic tumors PANC-1 and MiaPaca-2 cell lines (21).

Table 1. Effect of various concentrations of *Tripterygium wilfordii* methanolic extract on growth inhibition of two human embryonic liver WRL-68 and human pancreatic AsPC-1 cancer cells at 24-hour incubation

Concentrations	ASPC-1		WRL-68	
	GI%	SD	GI%	SD
400.000	51.003	1.050	68.619	5.050
200.000	64.082	2.985	79.892	5.014
100.000	80.864	5.863	88.788	7.448
50.000	94.290	0.821	97.646	2.750
25.000	95.949	0.904	95.988	1.678
12.500	94.869	0.291	95.178	0.406
6.250	94.830	0.707	95.679	0.406

The values are shown as mean ± SD (n=3) at the p-value of < 0.05

To the best of our knowledge, so far, no study has been conducted to investigate the anti-proliferative potential of the *T. wilfordii* plant on WRL-68 cell lines. However, there are experimental data supporting the potential anti-tumor activity of other medicinal plants against the human WRL-68 cell line.

Al-Saily and Omran (22) showed that *Peganum harmala* plant extract had a growth inhibitory effect on WRL-68 and MCF-7 cell lines. Similarly, Baharum, Akim (23) explained the cytotoxicity activity of *Theobroma cacao* extract against WRL-68 and MCF-7 cell lines. The results of a study conducted by Chung, Lee (24) revealed the anti-tumor activity of the *Glycyrrhiza uralensis* extract on WRL-68 and Hep3B cell lines.

3.2. Pharmacognostic Profile Analysis

The pharmacognostic profile of the methanolic extract of *T. wilfordii* achieved the occurrence of alkaloids, tannins, terpenoides, flavonoids, glycosides, and phenols as shown in table 2, and the mean of pH extracts was obtained at 5.5-6.

A huge array of therapeutic surveys of *T. wilfordii* may be assigned to its multiple varieties of compounds. Over 380 compounds have been reported for *T. wilfordii*. These compounds have also been tested in carcinoma cell lines, such as celastrol, and the blocked migration and invasion of prostate cancer cells (25); triptolide (TL) suppressed the proliferation of lung and pancreatic cancer cells (26, 27); nanoformulated a-mangostin and TL exhibited superior therapeutic effects in pancreatic ductal adenocarcinoma treatment (28); triptonide selectively activated the MEKK4/MKK4/p38 axis signaling pathway and stopped tumorigenicity in pancreatic cancer (29). Diterpenes have anti-cancer properties against Hela and L292 cell lines (30). The three triterpene components, namely triptotin G, wilforol A, and triptotin D, display a powerful cytotoxicity effect against leukemia and lung cancer cell lines (31).

Table 2. Pharmacognostic profile of the methanolic extract of *Tripterygium wilfordii*

Effective compounds	*Tripterygium wilfordii* extract
Alkaloids	+
Tannins	+
Terpenoides	+
Flavonoids	+
Glycosides	+
Phenols	+

It has been reported that hederagenin controlled the cytotoxic effect of cervical carcinoma cell (32); isoxanthohumol have versatile tumoricidal substances (33); B-sitosterol and gemcitabine suppressed epithelial-mesenchymal transition of pancreatic cancer cell lines through AKT/GSK-3B signaling pathways (34); kaempferol hindered pancreatic cancer cell growth and migration by suppressing pathways related to epidermal growth factor receptor (35). Alkaloids and polyglycoside are used for the treatment of a variety of autoimmune and inflammatory diseases, including rheumatoid arthritis, nephritis, Crohn's disease, and systemic lupus erythematosus (7, 29).

3.3. Evaluation of Gas Chromatography-Mass Spectrometry for Plant Extrxacts

As shown in figure 2, GC-MS of the chromatogram of *T. wilfordii* methanolic extract showed the presence of about four peaks. The molecular formula, molecular weight, retention time, and peaks area (%) are summarized in table 3.

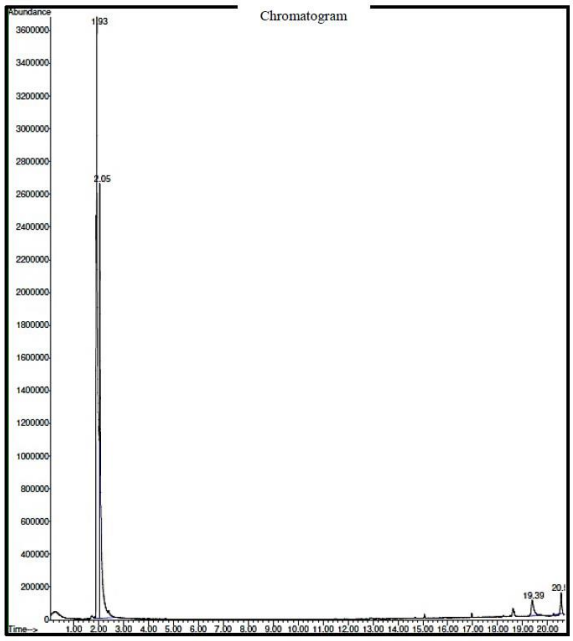

Figure 2. GC-MS chromatogram of the *Tripterygium wilfordii* methanolic extract

Table 3. Major pharmacognostic profiles obtained in the methanolic extract of *Tripterygium wilfordii*

N.	Retention time	Compound name	Area	Chemical formula	Nature of molecules
1	1.93	3-Oxobutanol	140247270	$C_4H_8O_2$	Phenol
2	1.93	Ethyl acetate	140247270	$C_4H_8O_2$	Carboxylic ester
3	1.93	Acetic acid ethyl ester	140247270	$C_4H_8O_2$	Carboxylic ester
4	1.93	Chlorbromuron	140247270	$C_9H_{10}BrClN_2O_2$	Organic compound
5	1.93	Monolinuron	140247270	$C_9H_{11}ClN_2O_2$	Organic compound
6	1.93	Metobromuron	140247270	$C_9H_{11}BrN_2O$	Organic compound
7	2.05	propene, 1-(methylthio)-, (E)-	86308698	C_4H_8S	Thioenol ethers
8	19.39	n-Hexadecanoic acid	6564703	$C_{16}H_{32}O_2$	Terpenoide
9	19.39	Tetradecanoic acid	6564703	$C_{14}H_{28}O_2$	Terpenoide
10	20.55	9-Octadecenoic acid, methyl ester	3264730	$C_{19}H_{36}O_2$	Terpenoide

The compounds in these peaks in the methanolic extract of *T. wilfordii* were mostly comprised of 3-Oxobutanol; ethyl acetate; acetic acid ethyl ester; chlorbromuron; monolinuron, metobromuron; propene, 1-(methylthio)-, (E)-; n-Hexadecanoic acid; tetradecanoic acid; and 9-Octadecenoic acid, methyl ester.

Nevertheless, these identified constituents are registered to have a number of pharmaceutical properties, such as anti-tumor, anti-microbial, anti-oxidant, anti-viral, anti-arthritis, anti-fungal, insecticidal, and other therapeutic potentials (36, 37). Moreover, 9-Octadecenoic acid, methyl ester, (E) compound has no bioactivity record to date, which needs to be explored in further studies.

In conclusion, *T. wilfordii* is a potent medicinal plant that showed a powerful anti-proliferative property, particularly against WRL-68 and AsPC-1 cell lines. These findings indicated that the anti-proliferative property of the methanolic extract of *T. wilfordii* leaves possessed potentially rich bioactive molecules, which holds a great promise as a future therapeutic agent in combating WRL-68 and AsPC-1 human cell lines. For the first time in the world, it has been shown that *T. wilfordii* has anti-proliferative properties against human liver WRL-68 cell lines. Further research analyses are required to discover new pathways to cancer therapy through new tumoricidal substances, which have been established by GC-MS.

Authors' Contribution

Study concept and design: N. T. H.
Acquisition of data: S. A. J.
Analysis and interpretation of data: A. K. A. A.
Drafting of the manuscript: A. K. A. A.
Critical revision of the manuscript for important intellectual content: S. A. J.
Statistical analysis: N. T. H.
Administrative, technical, and material support: N. T. H.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgment

The authors would like to thank Mustansiriyah University (www. uomustansiriyah.edu.iq) for its support to conduct the present study.

References

1. VdL-Guideline. 2021. Available from: https://www.Cancer.Org.
2. Sun CC, Bodurka DC, Weaver CB, Rasu R, Wolf JK, Bevers MW, et al. Rankings and symptom assessments of side effects from chemotherapy: insights from experienced patients with ovarian cancer. Support Care Cancer. 2005;13(4):219-27.
3. Seca AM, Pinto DC. Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. Int J Mol Sci. 2018;19(1):263.
4. Tijjani A, Ya S, Fi A, Iz K, Gm T, Li C. Isolation and Structural Elucidation of 20 Hydroxyecdysterone from Vitex doniana Sweet Stem bark (Black plum) Mustapha. Med Chem. 2017;7(3):828-31.
5. Yu J, DRISkO J, Chen Q. Inhibition of pancreatic cancer and potentiation of gemcitabine effects by the extract of Pao Pereira. Oncol Rep. 2013;30(1):149-56.
6. Liu L, Luo Y, Zhou M, Lu Y, Xing M, Ru Y, et al. Tripterygium agents for the treatment of atopic eczema: a Bayesian analysis of randomized controlled trials. Phytomedicine. 2019;59:152914.
7. Zhang J, Han G, Yu Z. Efficacy and safety of Tripterygium wilfordii Hook F preparations for the treatment of Crohn disease: a systemic review and meta-analysis protocol. Medicine. 2019;98(26).
8. Li J, Hao J. Treatment of neurodegenerative diseases with bioactive components of Tripterygium wilfordii. Am J Chin Med. 2019;47(04):769-85.
9. Lin N, Zhang Y-Q, Jiang Q, Liu W, Liu J, Huang Q-C, et al. Clinical Practice Guideline for Tripterygium Glycosides/Tripterygium wilfordii Tablets in the Treatment of Rheumatoid Arthritis. Front Pharmacol. 2021;11:2102.
10. Luo D, Zuo Z, Zhao H, Tan Y, Xiao C. Immunoregulatory effects of Tripterygium wilfordii Hook F and its extracts in clinical practice. Front Med. 2019;13(5):556-63.

11. Abid NBS, Rouis Z, Lassoued MA, Sfar S, Aouni M. Assessment of the cytotoxic effect and in vitro evaluation of the anti-enteroviral activities of plants rich in flavonoids. J Appl Pharm Sci. 2012;2(5):74.
12. Evans WC. Trease and evans' pharmacognosy E-book: Elsevier Health Sciences; 2009.
13. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media; 1998.
14. Zhao X, Liu Z, Ren Z, Wang H, Wang Z, Zhai J, et al. Triptolide inhibits pancreatic cancer cell proliferation and migration via down-regulating PLAU based on network pharmacology of Tripterygium wilfordii Hook F. Eur J Pharmacol. 2020;880:173225.
15. Ding X, Zhou X, Jiang B, Zhao Q, Zhou G. Triptolide suppresses proliferation, hypoxia-inducible factor-1α and c-Myc expression in pancreatic cancer cells. Mol Med Rep. 2015;12(3):4508-13.
16. Thani NAA, Keshavarz S, Lwaleed BA, Cooper AJ, Rooprai HK. Cytotoxicity of gemcitabine enhanced by polyphenolics from Aronia melanocarpa in pancreatic cancer cell line AsPC-1. J Clin Pathol. 2014;67(11):949-54.
17. Akasaka H, Mizushina Y, Yoshida K, Ejima Y, Mukumoto N, Wang T, et al. MGDG extracted from spinach enhances the cytotoxicity of radiation in pancreatic cancer cells. Radiat Oncol. 2016;11(1):1-11.
18. Rai V, Kumar A, Das V, Ghosh S. Evaluation of chemical constituents and in vitro antimicrobial, antioxidant and cytotoxicity potential of rhizome of Astilbe rivularis (Bodho-okhati), an indigenous medicinal plant from Eastern Himalayan region of India. BMC Complement Altern Med. 2019;19(1):1-10.
19. Rawa'a A, Hassawi DS, Ibaheem NK. Cytotoxic Activity of Taraxacum officinale Ethanolic Plant Extract against Human Breast Cancer (MCF-7) Cells and Human Hepatic (WRL-68) Cells. Iraqi J Cancer Med Genet. 2018;11(1-2018):16.
20. Wang X, Matta R, Shen G, Nelin LD, Pei D, Liu Y. Mechanism of triptolide-induced apoptosis: effect on caspase activation and Bid cleavage and essentiality of the hydroxyl group of triptolide. J Mol Med. 2006;84(5):405.
21. Phillips PA, Dudeja V, McCarroll JA, Borja-Cacho D, Dawra RK, Grizzle WE, et al. Triptolide induces pancreatic cancer cell death via inhibition of heat shock protein 70. Cancer Res. 2007;67(19):9407-16.
22. Al-Saily HM, Omran P. In Vitro Cytotoxicity of Total Alkaloid Extract from Peganum Harmala L. Seeds. Med Legal Update. 2020;20(1):1148-52.
23. Baharum Z, Akim AM, Taufiq-Yap YH, Hamid RA, Kasran R. In vitro antioxidant and antiproliferative activities of methanolic plant part extracts of Theobroma cacao. Molecules. 2014;19(11):18317-31.
24. Chung WT, Lee SH, Dai Kim J, Sung NS, Hwang B, Lee SY, et al. Effect of the extracts from Glycyrrhiza uralensis Fisch on the growth characteristics of human cell lines: Anti-tumor and immune activation activities. Cytotechnology. 2001;37(1):55-64.
25. Kuchta K, Xiang Y, Huang S, Tang Y, Peng X, Wang X, et al. Celastrol, an active constituent of the TCM plant Tripterygium wilfordii Hook. f., inhibits prostate cancer bone metastasis. Prostate Cancer Prostatic Dis. 2017;20(2):156-64.
26. Huang Y, Chen Z, Wang Y, Ba X, Huang Y, Shen P, et al. Triptolide exerts an anti-tumor effect on non‐small cell lung cancer cells by inhibiting activation of the IL‐6/STAT3 axis. Int J Mol Med. 2019;44(1):291-300.
27. Noel P, Hussein S, Ng S, Antal CE, Lin W, Rodela E, et al. Triptolide targets super-enhancer networks in pancreatic cancer cells and cancer-associated fibroblasts. Oncogenesis. 2020;9(11):1-12.
28. Feng J, Xu M, Wang J, Zhou S, Liu Y, Liu S, et al. Sequential delivery of nanoformulated α-mangostin and triptolide overcomes permeation obstacles and improves therapeutic effects in pancreatic cancer. Biomaterials. 2020;241:119907.
29. Zhang B, Meng M, Xiang S, Cao Z, Xu X, Zhao Z, et al. Selective activation of tumor-suppressive MAPKP signaling pathway by triptonide effectively inhibits pancreatic cancer cell tumorigenicity and tumor growth. Biochem Pharmacol. 2019;166:70-81.
30. Yao Z, Gao W, Takaishi Y, Duan H. Diterpenes from Tripterygium wilfordii and their anti-cancer activities. Chin Tradit Herb Drugs. 1994;(11).
31. Yang G, Li Y. Antitumor Triterpenoids from Tripterygium wilfordiiHook f. Chem Ind Forest Prod. 2006;26:19-22.
32. Fang L, Liu M, Cai L. Hederagenin inhibits proliferation and promotes apoptosis of cervical cancer CaSki cells by blocking STAT3 pathway. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2019;35(2):140-5.
33. Krajnović T, Kaluđerović GN, Wessjohann LA, Mijatović S, Maksimović-Ivanić D. Versatile antitumor potential of isoxanthohumol: Enhancement of paclitaxel activity in vivo. Pharmacol Res. 2016;105:62-73.
34. Cao Z-q, Wang X-x, Lu L, Xu J-w, Li X-b, Zhang G-r, et al. β-Sitosterol and gemcitabine exhibit synergistic anti-

pancreatic Cancer activity by modulating apoptosis and inhibiting epithelial–mesenchymal transition by deactivating Akt/GSK-3β signaling. Front Pharmacol. 2019;9:1525.

35. Lee J, Kim JH. Kaempferol inhibits pancreatic cancer cell growth and migration through the blockade of EGFR-related pathway in vitro. PloS One. 2016;11(5):0155264.

36. Adeoye-Isijola MO, Jonathan SG, Coopoosamy RM, Olajuyigbe OO. Molecular characterization, gas chromatography mass spectrometry analysis, phytochemical screening and insecticidal activities of ethanol extract of Lentinus squarrosulus against Aedes aegypti (Linnaeus). Mol Biol Rep. 2021;48(1):41-55.

37. Raafat K. Phytochemical analysis of Juglans regia oil and kernel exploring their antinociceptive and anti-inflammatory potentials utilizing combined bio-guided GC–FID, GC–MS and HPLC analyses. Rev Bras Farmacogn. 2018;28:358-68.