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Evaluation of Cytotoxic, Antioxidant and Antibacterial Activities of *Origanum dayi, Salvia palaestina* and *Bongardia chrysogonum* Plants Growing Wild in Jordan

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ABSTRACT

Ethnobotanical plants have been reported as promising antioxidant and anticancer agents. This study aims to investigate the potential antiproliferative, antioxidant and antibacterial activities of *Origanum dayi (O. dayi), Salvia palaestina* (*S. palaestina)* and *Bongardia chrysogonum* (*B. chrysogonum)*. The cytotoxicity of these plants against T47D cell line were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide (MTT) assay. The most potent plant (*O.* dayi) was selected and its apoptotic effect evaluated on T47D cell lines using flow cytometry. Also, the antioxidant effect of these plants was investigated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The minimum inhibitory concentration (MIC) assay was used to evaluate the antibacterial effect of the plants. *O. dayi* and *S. palaestina* exhibited cytotoxic activity against T47D cell line with IC_{50} of 250 ± 4.1 and 400 ± 5.2 µg/mL, respectively. Furthermore, *O. dayi* had the ability to induce apoptosis at low concentration and necrosis at high concentration against T47D cell line. Moreover, the free radical scavenging activity was significantly higher than ascorbic acid in only *B. chrysogonum*. *S. palaestina* and *O. dayi* exhibited inhibitory effects against all tested bacteria strain. This study confirms that the ethanol extracts of these plants possess antioxidant, and only *O. dayi*, *S. palaestina* have antibacterial as well as cytotoxic activity against T47D cell line at high concentration.

***Keywords*:** Antibacterial activity, Antioxidant potential, Cytotoxic, Apoptotic activity.

Introduction

Cancer is a major public health problem worldwide, and it is one of the leading cause of morbidity globally.[1-3] According to the international agency for research on cancer (IARC), there were 8.2 million cancer death worldwide in 2012, and by 2030 this burden is expected to grow to reach 13 million cancer death,[4] cancer cells are characterized by the unchecked division and survival of abnormal cells.[5] Therefore, there are ceaseless effort to search for new anticancer agents, since the chemotherapy might damage the normal cells while killing the cancer cell, which could lead to unfavourable side effect.[6,7]

Herbs are considered as alternative source for treatment since they have fewer side effects and less cost. In the developing countries, and Jordan in particular, a large portion of the population relies on different herbal natural plants such as *S. palaestina*, *O. dayi* and *B. chrysogonum*, for their primary healthcare as antioxidant, anti-inflammatory as well as in the treatment of cancer.[8-10] Herbal natural plants that are used in traditional medicine have a wide range of compounds; which can be used to treat infectious and chronic diseases.[11]

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In Jordan, it was reported that several medicinal plants have demonstrated antimicrobial activity against *Pseudomonas aeruginosa*, *Bacillus cereus, Staphylococcus aureus* and *Candida albicans*.[12]

In this study, we have investigated the cytotoxic effect of *O. dayi*, *S. palaestina* and *B. chrysogonum* against the T47D cell lines as well as the role of the *O. dayi* extract in the induction of apoptosis in T47D cell lines. In addition, we evaluated the antioxidant and antibacterial activities of these plants.

Materials and Methods

Plant materials

The aerial parts of the plants: *O. dayi*, *S. palaestina* and the corms of *B. chrysogonum* were collected in July, 2020. The plant species have been taxonomically identified at the Department of Biological Sciences in Jordan University. Voucher specimens of the collected plants were deposited at the University of Jordan.

O. dayi. Family name*:* Lamiaceae, site of collection*:* Al-karak. Voucher specimen*:* 4

S. palaestina. Family name*:* Lamiaceae, site of collection*:* Amman. Voucher specimen*:* 5

B. chrysogonum. Family name*:* Berberidaceae, site of collection*:* Amman. Voucher specimen*:* 7.

Preparation of plant extracts

Collected parts (aerial parts of *O. dayi* and *S. palaestina*) and the corms of *B. chrysogonum*) were air dried for approximately 6 weeks at room temperature (23 - 27°C) and powdered. 50 g of the powdered plant was dissolved in 500 mL of absolute ethanol (Solvent to sample ratio 1:10 w/v) by refluxing for 48 hours at 50°C.[13] The extract was then filtered through Whitman no. 1 filter paper. The filtrates were

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concentrated by using rotary evaporator at 60°C and subsequently left to completely dry.

Cell culture

Breast cancer cell line T47D was obtained from the US National Cancer Institute (NCI). Cells were cultured in Modified Eagle Medium (DMEM), supplemented with 10% heat inactivated fetal bovine serum (FBS), 20 μM L-glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin. Cells were maintained in incubator at 37℃ in a 5% CO_2 atmosphere with 95% humidity. After that, cells were harvested by trypsin- EDTA solution. Fibroblasts were used as normal cell lines.

Cell viability assay

Approximately 1×10^4 cells/mL were either left untreated or treated with 200, 100, 50, 25, 12.5 and 6.25 μg/mL of ethanol extract of the three plants separately. In order to assess cell viability, treated cells were incubated at 37°C for 72 h. Negative control wells contained only fresh media.[14] Control wells were cells with DMSO. Then, 20 μL of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to every well. The absorbance was measured at 570 nm and 630 nm using ELISA plate reader, and the difference in both readings was used for the analysis of results. The percentage of survival cells was detected by using the following formula:

% Survival = 100 – ((AC – AT) / AC) × 100).

IC_{50} values were detected as the concentrations that exhibited 50% inhibition of proliferation on the tested cell line. IC_{50} values were detected as the average of three replicates (Graph pad prism, version 5.02 statistical programes was used).[14,15]

Determination of apoptosis by flow cytometry

Approximately 5×10^5 cells were cultured in 25 cm^2 flask. ten flasks of T47D cells were prepared,[16] one flask was used as a control (untreated cells) and three flasks were treated with IC_{50}, IC_{75} and IC_{25} of ethanol extract of *O. dayi* and incubated for 72 h. Another three flasks were treated with IC_{50}, IC_{75} and IC_{25} of ethanol extract of *O. dayi* and incubated for 48 h. The last three flasks were also treated with IC_{50}, IC_{75} and IC_{25} of ethanol extract of *O. dayi* and incubated for 24 h. Then the number of cells were counted using hemocytometer according to the following formula: (Number of cell = average number of cells × 2 ×10000 × volume of cell suspension). Then 5×10^5 cells were collected into a sterile tube, the media were discarded and a fresh 500 μL of Ca^{+2}-enriched binding buffer was added to resuspend the cells, followed by the addition of 5 μL of Annexin V (APOAF, Sigma, St. Louis, MO)., and then incubated for 15 min in ice bath. Propidium iodide was added and incubated for 5 minutes. Three tubes of control were prepared, one left without staining, the second stained with annexin V, and the third stained with propidium iodide. The samples were analysed using BD FACSCanto II device, and FACSDiva software.[16]

Determination of Antioxidant activity using DPPH

The plant extracts were evaluated for their antioxidant potential by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[17] This assay was performed using the method described by Chan *et al*.,[18] with slight modification. Different dilutions of plant extracts were used in this assay (0.035 – 600 μg /mL). A solution of 0.1 mM DPPH was prepared in ethanol, and then 1 mL of this solution was mixed with 1 mL of sample solution and positive control solution separately. (Ascorbic acid was used as positive control at 0.1 nM). Then the optical density was measured at 517 nm using Spectrophotometer.

% DPPH scavenging = 100 × [(Abs Sample + DPPH) – (Abs Sample Blank)]/ [(Abs DPPH+ ethanol) – (Abs of ethanol)].

Determination of antibacterial activity

Bacteria were obtained from American type culture collection. In the present study bacteria were selected to cover Gram-negative bacteria (*E. aerogenes* ATCC 13048 and *E. coli* ATCC35218) and Gram-positive bacteria (*B. subtilis* ATCC6633 and *S. aureus* ATCC 25923). The antibacterial activity of ehanol extracts of *O. dayi*, *S. palaestina* and *B. chrysogonum* were estimated by determination of the MIC using microdilution method. MIC is the lowest concentration of an antimicrobial drug that inhibits visible growth. Various dilutions of the extracts were prepared (4.9 – 2500 μg/mL), and the broth that contains the plant extracts was inoculated with 50 μL bacteria suspention (1×10^6 CFU/mL).[19,20] The standard antibiotic tetracycline was used as a positive control. After incubation period, the bacterial growth was examined by observing the turbidity that is visually detected.[20,21]

Statistical analysis

One-way analysis of variance (ANOVA) was used for statistical analysis, the results were considered to be significant if $p < 0.05$. Data were expressed as the mean ± Standard Deviations (S.D).

Results and Discussion

In vitro cytotoxicity of ethanolic extracts of plants on T47D cell line

The cytotoxicity effect of the plant extracts on breast cancer cell line T47D was determined after 72 hours of incubation using MTT assay. Figure 1 shows that the ethanol extract of *B. chrysogonum* did not exhibit cytotoxic effect against T47D cell line. While ethanol extract of *O. dayi* exhibited highly cytotoxic potential against T47D cell line, followed by *S. palaestina*, none of these plants was equipotent to Doxorubicin (Table 1). Doxorubicin lacked selective cytotoxicity in fibroblasts, however, the *O. dayi* and *S. palaestina* extracts showed selectivity when they were tested on fibroblasts cell line (normal cells) (Figure 1 and Table 1). Recently, it was reported that Doxorubicin lacked selective cytotoxicity in fibroblasts cells which was in agreement with our results.[22] Nevertheless, the crude extracts, which contain mixture of different phytochemicals show cytotoxic selectivity.[22]

In the present study, the ethanol extract of *B. chrysogonum* did not show significant cytotoxicity against T47D cell lines. On the other hand, different species of *Salvia* have shown various antiproliferative effects against cancer cell lines. Previous studies demonstrated the moderate cytotoxic effects of *S. absconditiflora* against MCF7 cell lines.[23] In the present study the ethanol extract of *S. palaestina* have cytotoxic effects against T47D cell lines with IC_{50} = 400±5.2 μg/mL. This was in line with a study that investigated the antiproliferative activity of methanol extract of *S. palaestina* against choriocarcinoma (JEG-3) with IC_{50} of 377.6 μg/mL and endometrium adenocarcinoma (HEC-1A) with IC_{50} of 404.2 μg/mL.[24] However, *O. dayi* extract exerted higher antiproliferative effects against T47D than *B. chrysogonum* and *S. palaestina* extract on the same cell line. Therefore, we further studied the mechanism of action of *O. dayi* extract on T47D using flow cytometry**.**

Determination of apoptosis by flow cytometry for T47D cell lines treated with O. dayi

O. dayi exhibited the highest cytotoxicity against T47D cells among all the tested plants. Therefore, T47D cells were treated with different concentrations of *O. dayi*: 125 μg/mL **(**IC_{25}**)**, 250 μg/mL (IC_{50}) and 500 μg/mL (IC_{75}) of ethanol extract of *O. dayi* for 24, 48 and 72 hours, and then apoptosis was measured by using flow cytometry (Annexin V/Propedium Iodide (PI) staining). The detection of apoptosis is based on the early events of programmed cell death, where phosphatidylserine (PS), a phospholipid in cell membrane is translocated from the inner side of the cell membrane to the outer side. PS was detected by staining with a fluorescent Annexin V, which is a protein that is characterized by its high affinity for PS. This assay can differentiate between necrosis and apoptotic events. The results are displayed in Table 2. It appeared that following the 24 hours incubation time, at $IC_{25,}$ cells were heading toward apoptosis, while at $IC_{50,}$ cells were heading toward apoptosis and necrosis and at $IC_{75,}$ cells were heading towards necrosis in Table 2. After 48 hours, at $IC_{25,}$ low percentages of cells were heading toward apoptosis and necrosis,

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at $IC_{50;}$ cells were heading more toward apoptosis than necrosis. At $IC_{75,}$ cells were heading toward necrosis (Table 2). However, after 72 hour incubation time, at IC_{25}, IC_{50} and IC_{75}, cells were heading toward low percentages of apoptosis and high percentages of necrosis (Table 2). One of the mechanisms that is involved in cancer chemoprevention and treatment is the apoptotic mechanism.[25] In this study, the plant *O. dayi* exhibited cytotoxic effect against T47D cell line with IC_{50} = 250 ± 4.1 µg/mL. The analysis of annexin V- propidium (PI) assay for T47D treated with different concentrations of *O. dayi* showed a high extent of apoptosis compared to untreated cells (control). At low concentrations of ethanol extract of *O. dayi*, the bioactive compounds have induced apoptotic mechanism, while increase in the extract concentrations as well as the incubation time induced necrotic mechanism. It seems that at high concentrations (IC_{75}), the dose became toxic, leading the cells to follow necrosis mechanism. *O. dayi* has many compounds of essential oils. The majority of these oil compounds are (E) – sabinene hydrate, 1, 8-cineole, (E) - sabinene hydrate acetate, linalylacetate, terpinen-4-ol and α-terpineol.[26] Researchers have explored the apoptotic inducing mechanism of *O. dayi* on hepatocellular carcinoma HepG2 as well as on colon carcinoma Colo205.[27,28]

Antioxidant activity of plant extracts

ROS scavenging has been reported to be a very important antioxidant mechanism in inhibiting apoptosis and aging in the human body.[29] The radical scavenging capacities, as determined by DPPH assays for the ethanol extracts of the plants are shown in Figure 2. The scavenging effects of various extracts increased with increasing concentration in the range of 0.035-600 µg/mL. The IC_{50} value of DPPH scavenging activity for ascorbic acid was 0.5 µg/mL. and none of the investigated extracts could be identified as equally affective as ascorbic acid except *B. chrysogonum* extract (Table 3). Researchers have estimated the antioxidant activity of the oil in *S. palaestina* by DPPH method with IC_{50} of 2.3 mg/mL,[30] confirming the result of this study, which shows that the ethanol extract of *S. palaestina* has a scavenging activity on DPPH, with IC_{50} of 1.8 ± 0.2 µg/mL.

Determination of the antibacterial activity of O. dayi, S. palaestina, and B. chrysogonum extracts

The antibacterial activity of *O. dayi, S. palaestina,* and *B. chrysogonum* ethanol extracts was estimated using microdilution assay. Table 4 shows the MIC of various extracts. Results indicated that the ethanol extracts of *S. palaestina* and *O. dayi* have exhibited antibacterial potential at different concentrations. However, *B. chrysogonum* did not show inhibition of bacterial growth. Furthermore, none of the remaining extracts could be identified as equally affective as tetracycline.

In the present study, the antibacterial activity of *S. palaestina, O. dayi* and *B. chrysogonum* was investigated against various bacterial species: *E. aerogenes, E. coli, B. subtilis* and *S. aureus*. The ethanol extract of *S. palaestina* inhibited the growth of all bacterial species, which agree with a study that has tested the antibacterial activity of *S. palaestina* against *S. aureus* using disc diffusion method.[30] Flavonoids found in *S. palaestina*, is a group of the compounds that have demonstrated antibacterial potentials against a wide range of bacteria.[31] However, this study shows that the ethanol extract of *B. chrysogonum* did not exhibit antibacterial activity against *E. aerogenes, E. coli, B. subtilis* and *S. aureus*, which corresponds with a study that exhibited no antibacterial effect of methanol extract of *B. chrysogonum* on *S. aureus*.[20] No study to the best of our knowledge has been conducted on the antibacterial potential of *O. dayi*. However, in this study, the ethanol extract of *O. dayi* showed antibacterial activity against the tested bacterial species. This study suggests that the phytochemicals of *O. dayi* have antibacterial potential, by exhibiting growth inhibition of the tested gram-positive and negative bacteria.

Table 1: IC_{50} value (µg/mL) of the ethanol extract of the plants

Cell line	Plant extract (µg/ml)			
	B. chrysogonum	*O. dayi*	*S. palaestina*	Doxorubicin
T47D	NI	250 ± 4.1	400 ± 5.2	2.1 ± 1.3
Fibroblast	NI	NI	NI	0.9 ± 1.1

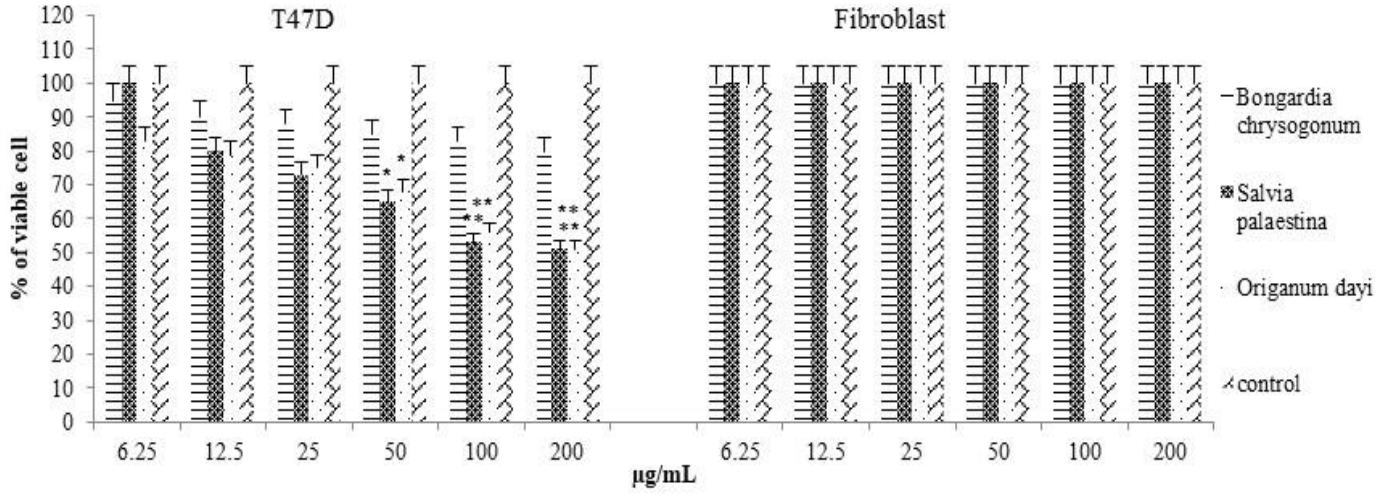

Figure 1: The cytotoxic effects of the crude ethanol extract of *B. chrysogonum, S. palaestina* and *O. dayi* on T47D cell line, and on Fibroblast. Cells were plated onto 96 well plates and treated with or without (control) different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL) of the extract for 72 h. The evaluation of cytotoxic activity is based on MTT assay. Data are representative of at least three independent experiments. The values are the mean of three dependent replicates ± SD. *: $p < 0.05$, **: $p < 0.01$ compared to control, ns: non-significant cytotoxicity. The result was analyzed using one-way ANOVA.

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Table 2: Flow cytometry of annexin V binding (FlTC-A) vs. propidium iodide (PI) staining (PI-A) in T47D cell line treated with IC_{50}, IC_{25} and IC_{75} of the ethanol extract of *O. dayi.*

Treatment	Q1(% of necrotic cells)	Q2 (% of late apoptotic cells)	Q4 (% of early apoptotic cells	Q3 (% of living cells) (control)
untreated T47D cells (control)	0.2%	0	0	99.7%
IC_{25} after 24 h incubation	7.3%	30%	3.8%	58.8%
IC_{50} after 24 h incubation	50%	24%	0.7%	23.7%
IC_{75} after 24 h incubation	86%	2.6%	0.1%	11.3
IC_{25} after 48 h incubation	18%	33.8%	0.4%	47.7%
IC_{50} after 48 h incubation	19.3%	7.4	2.9%	70.4%
IC_{75} after 48 h incubation	72.8%	9%	0.2%	17.9%
IC_{25} after 72 h incubation	32.3%	13.9%	2.7%	51.1%
IC_{50} after 72 h incubation	46.7%	19.4%	2.4%	31.5%
IC_{75} after 72 h incubation	58.5%	29.2%	0.2%	12.1%

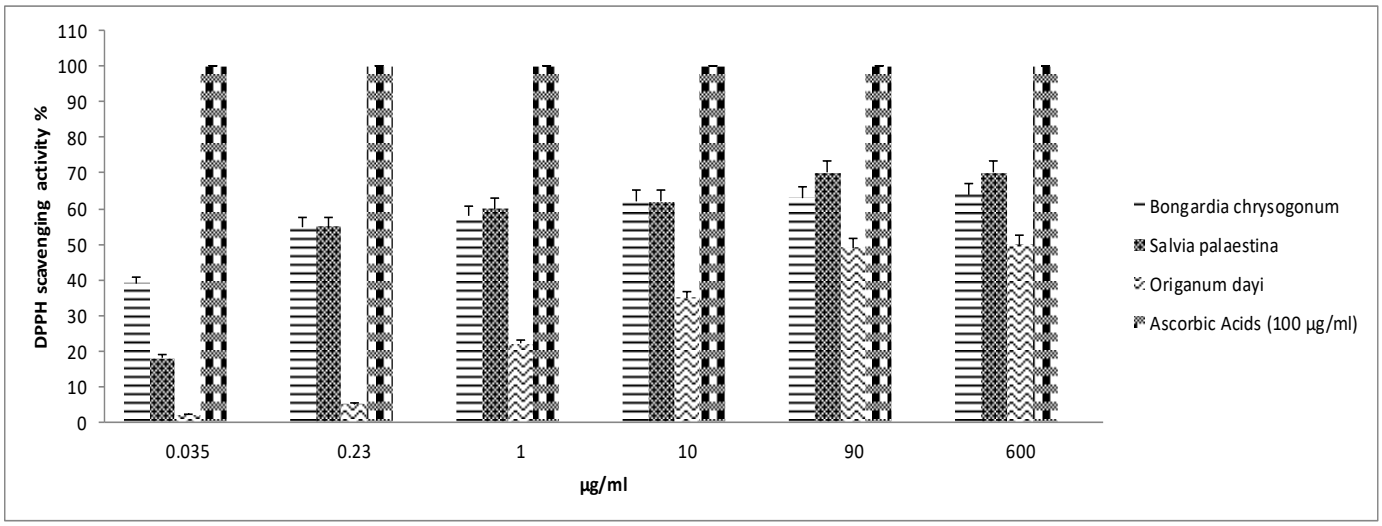

Figure 2: Antioxidant activity of various concentrations of the ethanol extracts of *B. chrysogonum. O. dayi* and *S. palaestina*
The results represent percentage of DPPH scavenging activity. Ascorbic acid was used as positive control at 0.1mM. Data are expressed as means of three measurements ± SD.

Table 3: The IC_{50} value for DPPH scavenging activity of the ethanol extracts of the plants

	Plant extract (µg/ml)			
	B. chrysogonum	*O. dayi*	*S. palaestina*	Ascorbic acid
IC_{50}	0.2 ± 1.4	181 ± 0.5	1.8 ± 0.2	0.5 ± 0.7

Results are mean ± SD (n = 3-4 independent replicates). IC_{50} values (concentration at which 50% of DPPH is scavenging in comparison to untreated sample).

Table 4: The MIC values of the plant ethanol extracts

Bacteria strains	MIC (µg/mL)			
	B. chrysogonum	***O. dayi***	***S. palaestina***	**Tetracycline**
S. aureus	NI	312.5	156.25	9.8
E. aerogenes	NI	625	625	9.8
E. coil	NI	156.2	156.2	9.8
B. subtilis	NI	625	625	78.1

Results are mean ± SD (n = 3-4 independent replicates). NI: corresponds to not inhibit over the concentration range tested (4.9-2500 µg/mL).

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Conclusion

This study demonstrated that the tested ethanol extracts of *O. dayi* and *S. palestina* possess antioxidant and antibacterial properties as well as cytotoxicity against T47D cell line at high concentration.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Qadir M, Ghalia B. Awareness survey about colorectal cancer in students of M. Phil Biotechnology at Bahauddin Zakariya University, Multan, Pakistan. Nov Appro in Can Study. 2018; 1(3).
2. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biol Res. 2017; 50(1):33.
3. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Front Microbiol. 2019; 10:911.
4. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends—an update. Cancer Epidemiol Prev Biomarkers. 2016; 25(1):16-27.
5. Yadav A and Mohite S. Cancer-A Silent Killer: An Overview. Asian J Pharm Res. 2020; 10(3):213-216.
6. Farhood B, Mortezaee K, Goradel NH, Khanlarkhani N, Salehi E, Nashtaei MS, Najafi M, Sahebkar A. Curcumin as an anti-inflammatory agent: Implications to radiotherapy and chemotherapy. J Cell Physiol. 2019; 234(5):5728-5740.
7. Mittra I, Pal K, Pancholi N, Shaikh A, Rane B, Tidke P, Kirolikar S, Khare NK, Agrawal K, Nagare H, Nair NK. Prevention of chemotherapy toxicity by agents that neutralize or degrade cell-free chromatin. Ann Oncol. 2017; 28(9):2119-2127.
8. Oran SA and Al-Eisawi D. Ethnobotanical survey of the medicinal plants in the central mountains (North-South) in Jordan. J Biodiv Env Sci. 2015; 6(3):381-400.
9. Zainab L and Hanan THA. An updated assessment on anticancer activity of screened medicinal plants in Jordan: Mini review. J Pharmacog and Phytochem. 2020; 9(5):55-58.
10. Oran S and Al-Eisawi D. Check-list of medicinal plants in Jordan. Dirasat. 1998; 25(2):84-112.
11. Mickymaray S. Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive Compounds against Clinically Important Pathogens. Antibiotics 2019; 8(4):257.
12. Zeidan R, Oran S, Khleifat K, Matar S. Antimicrobial activity of leaf and fruit extracts of Jordanian *Rubus sanguineus* Friv.(Rosaceae). Afr J Microbiol Res. 2013; 7(44):5114-5118.
13. Ling B, Michel D, Sakharkar MK, Yang J. Evaluating the cytotoxic effects of the water extracts of four anticancer herbs against human malignant melanoma cells. Drug Des Devel Ther. 2016; 10:3563.
14. Alqaraleh M, Kasabri V, Al-Othman N. Evaluation of Pancreatic and Extra Pancreatic Effects of Branched Amino Acids. Rom J Diabet Nutr Metab Dis. 2019; 26(2):199-209.
15. Alqaraleh M and Kasabri V. The Antiglycation Effect of Monomethyl Branched Chained Fatty Acid and Phytochemical Compounds and their Synergistic Effect on Obesity Related Colorectal Cancer Cell Panel. Rom J Diabet Nutr Metab Dis. 2019; 26(4):361-369.
16. Crowley LC, Marfell BJ, Scott AP, Waterhouse NJ. Quantitation of apoptosis and necrosis by annexin V binding, propidium iodide uptake, and flow cytometry. Cold Spring Harbor Protocols. 2016; 2016(11):087288.
17. Sirivibulkovit K, Nouanthavong S, Sameenoi Y. based DPPH assay for antioxidant activity analysis. Anal Sci. 2018; 34(7):795-800.
18. Chan E, Lim Y, Omar M. Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. Food Chem. 2007; 104(4):1586-1593.
19. Miski M, Ulubelen A, Johansson C, Mabry TJ. Antibacterial activity studies of flavonoids from Salvia palaestina. J Nat Prod. 1983; 46(6):874-875.
20. Assaf AM, Haddadin RN, Aldouri NA, Alabbassi R, Mashallah S, Mohammad M, Bustanji Y. Anti-cancer, anti-inflammatory and anti-microbial activities of plant extracts used against hematological tumors in traditional medicine of Jordan. J Ethnopharmacol. 2013; 145(3):728-736.
21. Abuhamdah S, Shatarat A, Al-Essa M, Al-Ameer H, Al-Olimat S, Chazot P. Spasmolytic and antimicrobial activities of crude extract of *Bongardia chrysogonum* L. tubers. Int J Pharmacol. 2017; 14(1):52-60.
22. Al-Tawarah NM, Qaralleh H, Khlaifat AM, Nofal MN, Alqaraleh M, Khleifat KM, Al-limoun MO, Al Shhab MA Anticancer and Antibacterial Properties of *Verthemia iphionides* Essential Oil/Silver Nanoparticles. Biomed Pharmacol J. 2020; 13(3):1175-1185.
23. Yıldırım I and Kutlu T. Anticancer agents: saponin and tannin. Int J Biol Chem. 2015; 9:332-340.
24. Fiore G, Nencini C, Cavallo F, Capasso A, Bader A, Giorgi G, Micheli L. In vitro antiproliferative effect of six Salvia species on human tumor cell lines. *In vitro* antiproliferative effect of six Salvia species on human tumor cell lines. Phytother Res. 2006; 20(8):701-703.
25. Cui Y, Pan M, Ma J, Song X, Cao W, Zhang P. Recent progress in the use of mitochondrial membrane permeability transition pore in mitochondrial dysfunction-related disease therapies. Mol Cell Biochem. 2020; 30:1-14.
26. Dudai N, Larkov O, Chaimovitsh D, Lewinsohn E, Freiman L, Ravid U. Essential oil compounds of *Origanum dayi* Post. Flavour Fragrance J. 2003; 18(4):334-337.
27. Bhatia D, Mandal A, Nevo E, Bishayee A. Apoptosis-inducing effects of extracts from desert plants in HepG2 human hepatocarcinoma cells. Asian Pacific J Trop Biomed. 2015; 5(2):87-92.
28. Solowey E, Lichtenstein M, Sallon S, Paavilainen H, Solowey E, Lorberboum-Galski H. Evaluating medicinal plants for anticancer activity. The Sci World J. 2014; 2014.
29. Hwang K-A, Hwang Y-J, Song J. Antioxidant activities and oxidative stress inhibitory effects of ethanol extracts from Cornus officinalis on raw 264.7 cells. BMC Comple Altern Med. 2016; 16(1):196.
30. Sabbobeh R, Hejaz H, Jahajha A, Al-Akhras S, Al-Jaas H, Abu-Lafi S. Antioxidant an antimicrobial activities of the leaf extract of Salvia palaestina. J Appl Pharm Sci. 2016; 6:76-82.
31. Pop A, Tofană M, Socaci SA, Pop C, Rotar AM, Salantă L. Determination of antioxidant capacity and antimicrobial activity of selected Salvia species. Bull.UASVM Food Sci Technol. 2016; 73:14-18.