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The Preliminary Study on Phytochemical Profile and Antioxidant and Cytotoxic Activities of Harmal (*Peganum harmala* L.)

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Abstract: Harmal (*Peganum harmala* L.) is a perennial plant that has come to the fore with its medicinal use since ancient times. In this study, the phytochemical profile of the methanol extract of harmal was characterized by HPLC and total phenolic and flavonoid amounts were determined by spectrophotometric methods. Additionally, antioxidant and cytotoxic (HT-29 (human colon cancer line), HeLa (human cervical cancer line), and HEK-293 (human embryonic kidney 293)) activities were investigated. Among 20 different phytochemical compounds screened, the presence of catechin (2.65±0.01 mg/g), coumarin (0.96±0.01 mg/g), ascorbic acid (0.91±0.02 mg/g), protocatechuic acid (0.85±0.01 mg/g), gallic acid (0.60±0.01 mg/g), hesperidin (0.24±0.01 mg/g), ferulic acid (0.06±0.01 mg/g), rutin (0.04±0.01 mg/g), and *trans*-cinnamic acid (0.03±0.01 mg/g) were detected. Total phenolic amount was found as 63.80±0.13 µg GAE/mg extract and total flavonoid amount was found as 13.50±0.36 µg QE/mg extract. Harmal methanol extract showed varying degrees of antioxidant activity in $ABTS^{\bullet+}$ (IC_{50}: 353.67±1.10 µg/mL), $DPPH^{\bullet}$ (17.54±0.45%), CUPRAC (absorbance: 0.41±0.02), phosphomolybdenum ($A_{0.50}$: 99.11±0.02 µg/mL), and metal chelating (38.47±0.77%) assays. Harmal methanol extract was low cytotoxic active on HT-29 cell line (> 800 µg/mL) and near-positive control cytotoxic active on HeLa cell line (IC_{50}: 45.84±0.95 µg/mL). These findings highlight harmal as a valuable reserve in the search for natural antioxidant and cytotoxic (on HeLa cell line) agents, especially with respect to the research focusing on possible pharmaceutical and food additive applications.

Keywords: Plant, HT-29 cell line, Methanol extract, HeLa cell line, HEK-293 cell line, HPLC.

Üzerlik (*Peganum harmala* L.) Bitkisinin Fitokimyasal Profili, Antioksidan ve Sitotoksik Aktiviteleri Üzerine Ön Çalışma

Öz: Üzerlik (*Peganum harmala* L.), antik çağlardan beri tıbbi kullanımı ile ön plana çıkan çok yıllık bir bitkidir. Bu çalışmada, üzerliğin metanol ekstresinin fitokimyasal profili HPLC ile karakterize edildi ve toplam fenolik ve flavonoid miktarları spektrofotometrik yöntemlerle belirlendi. Ayrıca, üzerliğin antioksidan ve sitotoksik (HT-29 (insan kolon kanseri hattı), HeLa (insan servikal kanser hattı) ve HEK-293 (insan embriyonik böbrek 293)) aktiviteleri araştırıldı. Taranan 20 farklı fitokimyasal bileşik arasında kateşin (2,65±0,01 mg/g), kumarin (0,96±0,01 mg/g), askorbik asit (0,91±0,02 mg/g), protokateşik asit (0,85±0,01 mg/g), gallik asit (0,60±0,01 mg/g), hesperidin (0,24±0,01 mg/g), ferulik asit (0,06±0,01 mg/g), rutin (0,04±0,01 mg/g) ve *trans*-sinnamik asit (0,03±0,01 mg/g) varlığı belirlendi. Toplam fenolik madde miktarı 63,80±0,13 µg GAE/mg ekstre ve toplam flavonoid miktarı 13,50±0,36 µg QE/mg ekstre olarak bulundu. Üzerlik metanol ekstresi, $ABTS^{\bullet+}$ (IC_{50}: 353,67±1,10 µg/mL), $DPPH^{\bullet}$ (%17,54±0,45), CUPRAC (absorbans: 0,41±0,02), fosfomolibden ($A_{0,50}$: 99,11±0,02 µg/mL) ve metal kelatlama (%38,47±0,77) yöntemlerinde değişen derecelerde antioksidan aktivite gösterdi. Üzerlik metanol ekstresi HT-29 hücre hattında (> 800 µg/mL) düşük sitotoksik aktiviteye sahipken, HeLa hücre hattında (IC_{50}: 45,84±0,95 µg/mL) pozitif kontrole yakın sitotoksik aktivite gösterdi. Bu bulgular, üzerliğin özellikle olası ilaç ve gıda katkı maddesi uygulamalarına odaklanan araştırmalar açısından doğal antioksidan ve sitotoksik (HeLa hücre hattına karşı) ajanların arayışında değerli bir rezerv olduğunu ortaya koymaktadır.

Anahtar kelimeler: Bitki, HT-29 hücre hattı, Metanol ekstresi, HeLa hücre hattı, HEK-293 hücre hattı, HPLC.

1. Introduction

Plants are hidden gems of bioactive compounds with a wide range of bioactive properties. These bioactive compounds produced in plants because of normal metabolic pathways are generally classified as secondary metabolites (Altemimi et al., 2017). Throughout history, mankind has relied on plants, which are the center of bioactive compounds, to address and eliminate many diseases (Davis & Choisy, 2024). These therapeutic benefits of plants have led to their inclusion in both traditional and modern medical practices from ancient times to the contemporary health system today. Medicines obtained from medicinal plants have different chemical and biological properties that make them remarkable in the field (Balkrishna et al., 2024). Their increasing global recognition is related to their capacity to supply natural solutions for diseases and contribute to the progress of health care. In determining the potential of a plant, not only the nutritional importance of that plant but also its medicinal properties should be properly recognized, thus providing a valuable contribution to general health and

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disease prevention (Alves & Rosa, 2007). Extraction of bioactive components of plants provides raw materials for the pharmaceutical and cosmetic industries. Biomedical research is based on comprehensive studies on the phytochemical profile and biological mechanisms of plant species or specific compounds found in plants. Thus, the research conducted is extremely important in identifying new biological resources that can be used for disease prevention and therapeutic interventions (Hasan et al., 2024).

Cancer is one of the leading causes of death worldwide. Despite great efforts to discover effective chemotherapy drugs, there are still unsolved toxicity and selectivity problems (Chunarkar-Patil et al., 2024). The toxicity of modern chemotherapy and the resistance of cancer cells to anti-cancer agents lead to the search for new methods for the treatment or prevention of this insidious disease (Liu et al., 2024). Plants are the basic building blocks leading to the development of new anti-cancer drugs. In the last 20 years, more than a quarter of new drug molecules have been obtained from plant sources and the other quarter are their chemically modified derivatives (Grigalius & Petrikaite, 2017). Today, approximately half of the approved drugs are of natural origin (Asma et al., 2022).

Reactive oxygen species (ROS) is a collective term often used for unstable and reactive molecules derived from oxygen during cellular metabolism. Cells can produce ROS through various endogenous and exogenous mechanisms (Gu et al., 2024). Excessive ROS production leads to oxidative stress, which can have toxic effects on DNA, proteins, lipids, and other biomolecules, resulting in cell apoptosis or ferroptosis (Hayes et al., 2020). Furthermore, increased oxidative stress may contribute to tumor progression by directly oxidizing macromolecules or aberrant redox signaling resulting from oxidative stress, triggering tumor development (An et al., 2024). Studies have shown that high ROS levels may increase the risk of cancer when antioxidants are insufficient to protect cells from oxidative stress. In this context, the use of antioxidants in the treatment of cancer is an attractive strategy, as oxidative stress plays an important role in carcinogenesis and cancer progression (Ladas et al., 2004; Luo et al., 2022).

Harmal (*Peganum harmala* L.) belongs to *Zygophyllales* genus and Zygophyllaceae family. Harmal is a medicinal plant that grows naturally in South Asia and North Africa in general and used in traditional medicine as a decoction, powder and infusion for diarrhea, abortion, asthma, lumbago, and other ailments in Türkiye, Syria, Iran, Pakistan, India, Egypt, and Spain. This plant species is also known for its specific polyphenolic and alkaloid compounds (Asgarpanah & Ramezanloo, 2012). Antibacterial, antileishmanial, antiviral, antidiabetic, antitumor, antioxidant, insecticidal, cytotoxic, antifungal, hepatoprotective, and antinociceptive effects have been emphasized in earlier studies (Moloudizargari et al., 2013). This study was designed to investigate the phytochemical profile and bioactive properties of the methanol extract of harmal. For this purpose, the phytochemical profile of harmal was characterized by HPLC and the total amounts of phenolic and flavonoid were determined. In addition, antioxidant and cytotoxic activities of harmal were also tested.

2. Material and Method

2.1. Plant Material and Extraction

Harmal (*Peganum harmala* L.) was purchased from the local herbal market of Konya-Türkiye. The aerial parts of the plant sample, which were cut into small pieces, were extracted with methanol for 24 h at room temperature according to the maceration method. After the mixture was filtered, the residue was extracted with methanol 3 more times using the same method. All the methanol phases obtained were combined and evaporated in a rotary evaporator. Thus, methanol extract was obtained.

2.2. Phytochemical Profile

Phytochemical profile of harmal methanol extract was characterized by using HPLC. C_{18} column (5 µm, 250 mm × 4.6 mm i.d) (Ace Generix reverse phase) was preferred to sperate phytochemicals. The oven temperature was 30°C, solvent flow rate was 0.8 mL/min, and sample injection volume was 10 µL. The mobile phases consisted of 0.1% phosphoric acid in water (A) and acetonitrile (B). The elution gradient was as follows: 0-7 min, 0-17% B; 7-20 min, 17-15% B; 20-24 min, 15-20% B; 24-28 min, 20-25% B; 28-30 min, 25-30% B; 30-32 min, 30-40% B; 32-36 min, 40-50% B; 36-40 min, 50-70% B; 40-45 min, 70-17% B. Detection was operated via photodiode array detector (PDA) at 280 nm wavelength. Retention times and UV data were matched with commercial standards to identify the compounds. Three parallel analyses were practiced. The known concentrations of different standard compounds i.e. rutin, naringin, ascorbic acid, hesperidin, gallic acid, flavone, protocatechuic acid, catechin, *p*-hydroxy benzoic acid, vanillic acid, gentisic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, neohesperidin, resveratrol, quercetin, coumarin, *trans*-cinnamic acid, and alizarin were injected and calibration curves were obtained for the quantitative analysis of the phytochemical compounds (Deveci et al., 2023).

2.3. The Amounts of Total Phenolic and Total Flavonoid

Folin Ciocalteu assay was applied to experience of total phenolic amount of harmal methanol extract (Slinkard & Singleton, 1977). 1 mL harmal methanol extract, positive control or control solution, 46 mL distillated water, and 1 mL FCR were mixed. After 3 min, 3 mL Na_2CO_3 solution was added and the mixture was shaken for 2 h at room temperature. The absorbance was read at 760 nm. The result was given as µg gallic acid equivalent (GAE).

Aluminium nitrate assay was applied to experience of total flavonoid amount of harmal methanol extract (Park et al., 1997). 100 µL harmal methanol extract, positive control or control solution, 4.8 mL ethanol, 100 µL potassium acetate and 100 µL aluminium nitrate solution were added. The mixture was shaken for 40 min at room temperature. The absorbance was read at 415 nm. The result was given as µg quercetin equivalent (QE).

2.4. Antioxidant Activity

Antioxidant activity was investigated by DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging, phosphomolybdenum, cupric reducing antioxidant capacity (CUPRAC), and metal chelating assays. Ascorbic acid, α-tocopherol, BHA, and

EDTA were used as positive controls. The results were presented as inhibition percentages (%) and absorbance at 800 µg/mL concentration, IC_{50} and $A_{0.50}$ values.

2.4.1. DPPH• Scavenging Activity

DPPH• scavenging assay was applied to experience of antioxidant activity of harmal methanol extract as stated in our prior study (Deveci et al., 2024). 40 µL harmal methanol extract, control or positive control solution and 160 µL DPPH• solution were mixed and the absorbance was read at 517 nm after 30 min.

2.4.2. ABTS•+ Scavenging Activity

ABTS•+ scavenging assay was applied to experience of antioxidant activity of harmal methanol extract as stated in our prior study (Deveci et al., 2024). 40 µL harmal methanol extract, control or positive control solution and 160 µL ABTS•+ solution were mixed and the absorbance was read at 734 nm after 10 min.

2.4.3. CUPRAC Activity

CUPRAC assay was applied to experience of antioxidant activity of harmal methanol extract as stated in our prior study (Deveci et al., 2024). 50 µL $CuCl_2$, 60 µL NH_4Ac buffer, 50 µL neocuproine, and 40 µL harmal methanol extract, control or positive control solution were mixed. The absorbance was read at 450 nm after 1 h.

2.4.4. Metal Chelating Activity

Metal chelating assay was applied to experience of antioxidant activity of harmal methanol extract as stated in our prior study (Deveci et al., 2024). 80 µL harmal methanol extract, control or positive control solution, 40 µL $FeCl_2$, and 80 µL ferene were mixed and the absorbance was read at 593 nm.

2.4.5. Phosphomolybdenum Reducing Antioxidant Power

Phosphomolybdenum assay was applied to experience of antioxidant activity of harmal methanol extract as stated in our prior study (Deveci et al., 2024). 300 µL harmal methanol extract, control or positive control solution and 3 mL the reagent solution (H_2SO_4, Na_3PO_4, $(NH_4)_6Mo_7O_{24}$) were incubated for 90 min at 95°C. When the mixture cooled to the room temperature, the absorbance was read at 695 nm.

2.5. Cytotoxic Activity

Alamar blue assay was applied to experience of cytotoxic activity of harmal methanol extract on HT-29 (human colon cancer line), HeLa (human cervical cancer line), and HEK-293 (human embryonic kidney 293) (Yılmaz, 2022). Cells previously stored at -80°C were thawed in a water bath, centrifuged, and then placed in the growth medium. In the incubation of the cells, DMEM (10% FBS, 1% penicillin-streptomycin, 0.01% gentamicin) and RPMI (10% FBS, 1% penicillin-streptomycin, 0.01% gentamicin) media were used in a 5% CO_2 atmosphere at 37°C. As a result of obtaining active cells that reached sufficient capacity, the cells were transferred to the transition media, washed with PBS, and the cells were separated from the surface by trypsinization. The obtained cell pellets were diluted in the appropriate medium and then transferred to fresh cell culture dishes. Cytotoxic activity was performed based on Alamar Blue assay. 37°C and 5% CO_2 environment was used in the incubation of cell lines seeded in 96-well plates. After removal of the growth medium, harmal methanol extract, control or positive control was added to each well and incubation continued. Alamar Blue® reagent was added after 18 h and incubated for 4 h. Absorbance was read at 570 nm and 600 nm. Cisplatin and doxorubicin were used as positive controls. The results were presented as cell growth (%) and IC_{50} values.

2.6. Statistical analysis

All results were the average of three parallel sample measurements and presented as the mean ± S.E. (standard error). Student's *t* test was used to analyze significant differences and *p* values <0.05 were accepted as significant.

3. Results

Phytochemical profile of harmal methanol extract was screened by HPLC. The HPLC chromatogram of the standards is given in Figure 1 and harmal methanol extract in Figure 2. Table 1 shows the analyzed and identified compounds. The presence of catechin (2.65±0.01 mg/g), coumarin (0.96±0.01 mg/g), ascorbic acid (0.91±0.02 mg/g), protocatechuic acid (0.85±0.01 mg/g), gallic acid (0.60±0.01 mg/g), hesperidin (0.24±0.01 mg/g), ferulic acid (0.06±0.01 mg/g), rutin (0.04±0.01 mg/g), and *trans*-cinnamic acid (0.03±0.01 mg/g) were detected in harmal methanol extract.

Total phenolic and flavonoid amounts of harmal methanol extract were spectrophotometrically tested. As seen in Table 2, when total phenolic amount was found as 63.80±0.13 µg GAE/mg extract, total flavonoid amount was found as 13.50±0.36 µg QE/mg extract.

Since antioxidants exhibit various mechanisms of action, five different methods (DPPH• and ABTS•+ scavenging, phosphomolybdenum, CUPRAC, metal chelating) were used here to better determine antioxidant activity. The results are given in Table 3. Harmal methanol extract was found as antioxidant active in all assays with IC_{50} value as 353.67±1.10 µg/mL in ABTS•+ scavenging assay, $A_{0.50}$ value as 99.11±0.02 µg/mL in phosphomolybdenum assay, absorbance as 0.41±0.02 in CUPRAC assay, inhibition values as 17.54±0.45% and 38.47±0.77% in DPPH• scavenging and metal chelating assays at 800 µg/mL, respectively.

Cytotoxic activity of harmal methanol extract was investigated according to Alamar blue assay. The cell growth values of HT-29, HeLa, and HEK-293 cell lines attained from treated different concentrations of harmal methanol extract and the positive controls are represented in Figure 3. The dose-dependent inhibition was obtained in all cancer lines. Table 4 shows the IC_{50} values for harmal methanol extract and positive controls. The cell growth value of harmal methanol extract was recorded as 59.51±2.24% on HT-29 cell line, 0.85±0.20% on HeLa cell line, and 88.33±1.26% on HEK-293 cell line at 800 µg/mL. When harmal methanol extract was found as very low active on HT-29 cell line with IC_{50} > 800 µg/mL, harmal methanol extract indicated near-positive control (IC_{50}: 31.02±0.05 µg/mL for cisplatin) cytotoxicity on HeLa cell line with IC_{50} of 45.84±0.95 µg/mL.

Table 1. Phytochemical profile (mg/g extract) of harmal methanol extract

Peak number	Compounds	Retention time (min)	Harmal
1	Ascorbic acid	3.372	0.91±0.02
2	Gallic acid	4.191	0.60±0.01
3	Protocatechuic acid	5.693	0.85±0.01
4	Catechin	6.518	2.65±0.01
5	*p*-Hydroxy benzoic acid	8.573	nd
6	Vanillic acid	9.722	nd
7	Gentisic acid	10.355	nd
8	*p*-Coumaric acid	17.214	nd
9	Rutin	19.084	0.04±0.01
10	Ferulic acid	20.223	0.06±0.01
11	Naringin	27.374	nd
12	*o*-Coumaric acid	28.686	nd
13	Neohesperidin	29.581	nd
14	Coumarin	30.805	0.96±0.01
15	Resveratrol	32.399	nd
16	Quercetin	34.732	nd
17	*trans*-Cinnamic acid	35.603	0.03±0.01
18	Hesperidin	36.702	0.24±0.01
19	Alizarin	38.661	nd
20	Flavone	40.769	nd

nd: Not detected.

Table 2. Total phenolic and flavonoid amounts of harmal methanol extract[a]

	Total phenolic amount (µg GAE/mg extract)[b]	Total flavonoid amount (µg QE/mg extract)[c]
Harmal	63.80±0.13	13.50±0.36

[a] Values state the means ± S.E. of three repetitions.

[b] GAE, gallic acid equivalents. Absorbance=0.0077[GAE (µg)]-0.007 (r^2, 0.9995).

[c] QE, quercetin equivalents. Absorbance=0.0154[QE (µg)]-0.0543 (r^2, 0.9998).

Table 3. Antioxidant activity of harmal methanol extract[a]

		Harmal	α-Tocopherol[e]	BHA[e]	Ascorbic acid[e]	EDTA[e]
$ABTS^{\bullet+}$	Inhibition (%)[b]	75.74±0.47	94.96±0.53	95.89±0.10	90.70±0.04	NT[f]
	IC_{50}[c]	353.67±1.10	38.51±0.54	11.82±0.18	5.24±0.18	NT[f]
$DPPH^{\bullet}$	Inhibition (%)[b]	17.54±0.45	96.12±0.42	96.05±0.08	89.65±0.03	NT[f]
	IC_{50}[c]	>800	37.20±0.41	19.80±0.36	6.68±0.94	NT[f]
CUPRAC	Absorbance[d]	0.41±0.02	2.93±0.16	3.50±0.04	3.42±0.01	NT[f]
	$A_{0.50}$[c]	>800	89.47±0.87	24.51±0.47	20.67±0.01	NT[f]
Phosphomolybdenum	Absorbance[d]	1.68±0.03	NT[f]	NT[f]	3.65±0.01	NT[f]
	$A_{0.50}$[c]	99.11±0.02	NT[f]	NT[f]	13.66±0.19	NT[f]
Metal chelating	Inhibition (%)[b]	38.47±0.77	NT[f]	NT[f]	NT[f]	96.30±0.11
	IC_{50}[c]	>800	NT[f]	NT[f]	NT[f]	3.50±0.44

[a] Values represent the means ± S.E. of three parallel measurements ($p < 0.05$); [b] Inhibition (%) at 800 µg/mL concentration; [c] Results were given as µg/mL; [d] Absorbance at 800 µg/mL concentration; [e] Positive controls; [f] NT: not tested.

Table 4. Cytotoxic activity of harmal methanol extract[a]

	HT-29[b]	HeLa[b]	HEK-293[b]
Harmal	>800	45.84±0.95	>800
Cisplatin[c]	14.75±0.87	31.02±0.05	NT[d]
Doxorubicin[c]	15.56±0.96	19.78±0.02	NT[d]

[a] Values represent the means ± S.E. of three parallel measurements ($p < 0.05$); [b] IC_{50} results were given as µg/mL; [c] Positive controls; [d] NT: not tested.

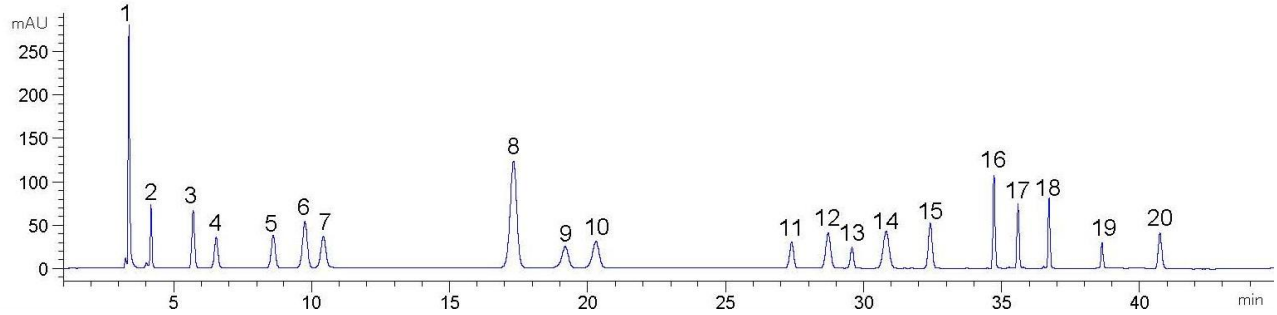

Figure 1. HPLC chromatogram of the standards

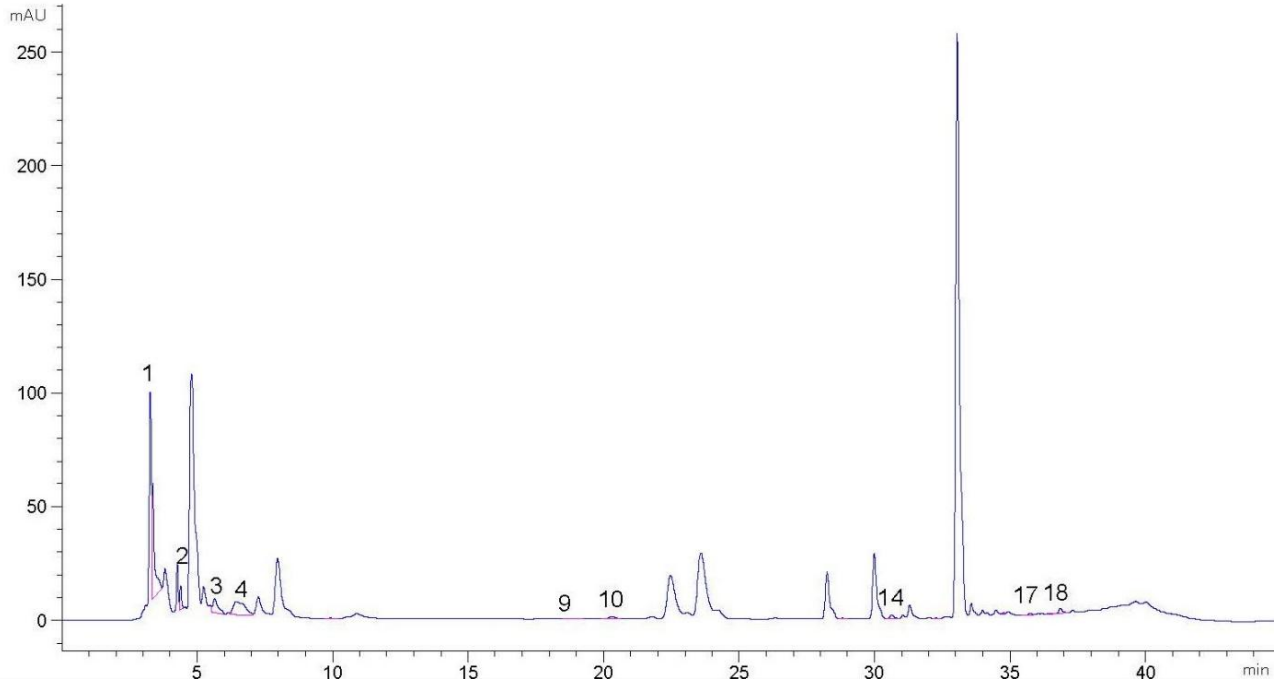

Figure 2. HPLC chromatogram of harmal methanol extract

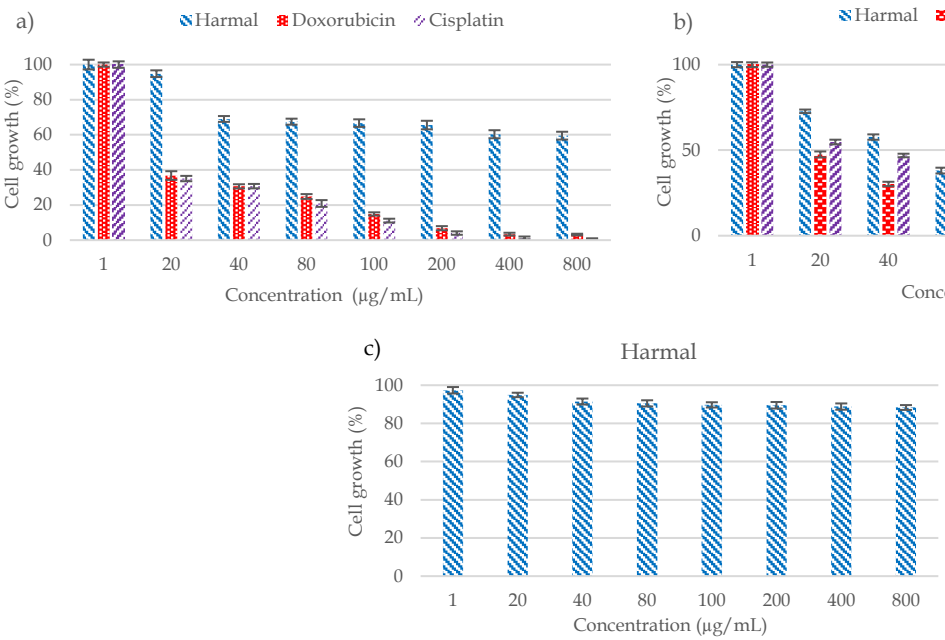

Figure 3. The cell growth values (%) of harmal methanol extract a) HT-29 cell line b) HeLa cell line c) HEK-293 cell line

4. Discussion

Phytochemical profile of harmal methanol extract was screened by HPLC. Among 20 phytochemical compounds analyzed, the presence of catechin, coumarin, ascorbic acid, protocatechuic acid, gallic acid, hesperidin, ferulic acid, rutin, and *trans*-cinnamic acid were detected. As

similar to our findings, gallic acid (0.02%), ascorbic acid (2.84%), quercetin (14.82%), butylated hydroxytoluene (0.41%), chlorogenic acid (0.26%), kaempferol (0.66%), hesperidin (2.04%), folic acid (3.33%), β-carotene (0.32%), naringenin (0.27%), hydroxy coumarin (0.10%), coumaric acid (70.61%), vanillin (1.77%), maleic acid (2.25%), and benzoic acid (0.23%) were identified in the butanol extract of harmal seeds from Algeria by UPLC-ESI-MS-MS (Kemel et al., 2024). In line with the results here, gallic acid (4.660 ppm), quercetin (2.747 ppm), caffeic acid (12.280 ppm), vanillic acid (13.253 ppm), chlorogenic acid (15.847 ppm), syringic acid (16.740 ppm), *p*-coumaric acid (17.767 ppm), *m*-coumaric acid (20.580 ppm), ferulic acid (22.280 ppm), cinnamic acid (25.133 ppm), and sinapic acid (26.060 ppm) were revealed as the phenolic acids in the leaves of harmal (Punjap-Pakistan) water extract by HPLC (Nazir et al., 2024). The synergic mixture (a mixture containing water, ethanol, acetone, chloroform, and hexane extracts) of harmal leaves from Pakistan was characterized by HPLC. Consistent with our results, the presence of ascorbic acid (261.97 µg/mL), caftaric acid (258.47 µg/mL), rosmeric acid (270.12 µg/mL), kaempferol (255.33 µg/mL), gallocatechin (270.03 µg/mL), caffeic acid (263.30 µg/mL), 3,4-*O*-dicaffeoylquinic acid (264.59 µg/mL), chrysin (268.14 µg/mL), catechin (270.17 µg/mL), chlorogenic acid (276.46 µg/mL), sinapic acid hexoside (327.41 µg/mL), kaempferol-*O*-dirhamnoside (494.80 µg/mL), chicoric acid (448.52 µg/mL), orientin (803.10 µg/mL), chebulic acid (503.83 µg/mL), malvidin-3-*O*-glucoside (538.18 µg/mL), and di-*O*-methyl ellagic acid (515.47 µg/mL) were observed (Kamran et al., 2024). Protocatechuic (51.6 mg/100 g), hydrocaffeic (199.4 mg/100 g), caffeic (20.6 mg/100 g), and rosmarinic (18.9 mg/100 g) acids, and luteolin-7-glucoside (713.5 mg/100 g) were characterized in the methanol extract of harmal leaves (from Saudi Arabia) by LC (Elansary et al., 2020a). In a different study on harmal seeds from Türkiye, interestingly, catechin (1.32 µg/g), luteolin (0.20 µg/g), and naringenin (0.38 µg/g) were identified in harmal hexane extract; only epicatechin (9.41 µg/g) in harmal ethanol extract; epicatechin (67.34 µg/g), naringin (0.17 µg/g), luteolin (0.1 µg/g), and naringenin (0.58 µg/g) in harmal water extract by HPLC (Gür et al., 2018). The basis of the differences between the findings here and the literature are not only the changes in the collection areas of the plant but also the agricultural, geographical, and seasonal factors that affect the phytochemical composition (Liu et al., 2022).

Phenolic compounds constitute the most important phytochemical compound class of plants due to the scavenging effects of hydroxyl groups on free radicals. These properties of phenolic compounds directly contribute to the antioxidant activities of plants (Tosun et al., 2009). Total phenolic and flavonoid amounts of harmal methanol extract were spectrophotometrically tested. When total phenolic amount of harmal methanol extract was found as 63.80±0.13 µg GAE/mg extract, total flavonoid amount was found as 13.50±0.36 µg QE/mg extract. The current findings are consistent with those of Mazandarani et al. (2012), who found 61.55 mg GAE/g extract of total phenolic content and 42.21 mg QE/g extract of total flavonoid content in the ethanol extract of harmal seeds from Iran. The study of Abbas et al. (2021) showed that harmal fruits from Pakistan had higher amounts of total phenolic compounds in dichloromethane (106.0 mg GAE/g), methanol (371.4 mg GAE/g), and 70% methanol (142.3 mg GAE/g) extracts and lower amounts of total flavonoid compounds in dichloromethane (0.31 mg QE/g), methanol (1.3 mg QE/g), and 70% methanol (0.81 mg QE/g) extracts than our findings. The higher amounts of total phenolic (144.97, 197.23, 237.23, and 111.25 µg GAE/mg, respectively) and flavonoid (43.62, 57.98, 82.58, and 22.36 µg QE/mg, respectively) compounds were reported in the methanol extract and butanol, ethyl acetate, and chloroform fractions of harmal seeds from Algeria (Nait Marzoug et al., 2023).

Herein, antioxidant activity of harmal methanol extract was analyzed by five different complementary methods (DPPH$^{\bullet}$ scavenging, ABTS$^{\bullet+}$ scavenging, CUPRAC, phosphomolybdenum, and metal chelating methods). Harmal methanol extract was found to be a high antioxidant active in all assays. Coumarins have been included in pharmaceutical products with their versatile antithrombotic, anti-inflammatory, antioxidant, antimicrobial, antiviral, anti-cancer, and neuroprotective pharmacological profiles and in perfumes and food products with their odor and flavor enhancing profiles (Elmusa & Elmusa, 2024). Coumarins have been reported to be promising super antioxidants for many synthetic antioxidant drugs, especially those that act by stopping DNA oxidation and scavenging radicals (Wang et al., 2020). Catechin is an important active antioxidant compound that acts by stopping reactive oxygen species, increasing antioxidant enzymes, inhibiting pro-enzymes involved in oxidative stress or chelating metals, in addition to its other bioactive abilities (Sheng et al., 2023). Ascorbic acid is a promising nutraceutical found naturally in plants and foods, exhibiting a wide range of medical or health benefits. It is a powerful antioxidant that protects cell membranes, DNA, and tissues from oxidative damage. Ascorbic acid interacts with free oxygen ions, superoxide ions, and hydroxyl ions with free radical properties, thereby stopping the formation of inflammation, carcinogens, and other factors that aggravate photoaging in the skin. It plays a critical role in maintaining health and fighting diseases and infections, especially with its prominent antioxidant properties (Ali et al., 2024). Multifaceted *in vivo* and *in vitro* studies have emphasized that the excellent antioxidant activity of protocatechuic acid is based on its ability to increase endogenous antioxidant enzyme activity and radical scavenging (Zhang et al., 2021). This antioxidant activity exhibited by harmal may be the result of a synergy of the high amounts of these compounds and other compounds identified in its content. In previous literature studies, antioxidant effect of harmal was mainly examined according to the radical scavenging methods. The similar DPPH$^{\bullet}$ scavenging activity was stated in harmal aerial parts (from Tunisia) methanol extract with IC_{50} value of 6 mg/mL and moderate antioxidant activity was also described in harmal petroleum ether (IC_{50}: 1.25 mg/mL), chloroform (IC_{50}: 3.25 mg/mL), ethyl acetate (IC_{50}: 0.850 mg/mL), and butanol (IC_{50}: 0.350 mg/mL) extracts (Edziri et al., 2010). It has been informed that the ethanol extract of harmal seeds (from Iran) exhibited antioxidant activity in DPPH$^{\bullet}$ scavenging (IC_{50}: 53.64 mg/mL), reducing power (IC_{50}: 84.75 mg/mL) and total antioxidant capacity (IC_{50}: 17.34 mg/mL) assays, supporting our results (Mazandarani et al., 2012). Higher DPPH$^{\bullet}$ scavenging activity was reported

in harmal fruits (from Pakistan) dichloromethane (IC_{50}: 146 µg/mL), methanol (IC_{50}: 49 µg/mL), and 70% methanol (IC_{50}: 69 µg/mL) extracts. Also, in the same study, harmal dichloromethane, methanol, and 70% ethanol extracts were reported as antioxidant active in FRAP (IC_{50}: 9.2, 39, 19.2 µg/mL) and H_2O_2 (25, 66, 43 %) assays (Abbas et al., 2021). The higher antioxidant activity was reported by Kemel et al. (2024) who observed that the butanol extract of harmal seeds from Algeria had 8.09 µg/mL IC_{50} value in $ABTS^{\bullet+}$ scavenging assay, 30.87 µg/mL IC_{50} value in $DPPH^{\bullet}$ scavenging assay, and 216.1 µg/mL OD_{50} value in FRAP assay. The methanol extract and butanol, ethyl acetate and chloroform fractions of harmal seeds from Algeria were investigated for antioxidant activity by using eight different assays. Harmal methanol extract and fractions were described as higher antioxidant than our results in $DPPH^{\bullet}$ scavenging (IC_{50}: 92.02-187.57 µg/mL), $ABTS^{\bullet+}$ scavenging (IC_{50}: 10.22-35.02 µg/mL), CUPRAC ($A_{0.50}$: 90.57-187.39 µg/mL), reducing power ($A_{0.50}$: 87.83-158.07 µg/mL), phenanthroline ($A_{0.50}$: 3.78-6.45 µg/mL), galvinoxyl radical scavenging (IC_{50}: 45.93-305.59 µg/mL), β-carotene bleaching (IC_{50}: 27.21-70.93 µg/mL), and ferrous ion chelating (> 800 µg/mL) assays (Nait Marzoug et al., 2023).

Cytotoxic activity of harmal methanol extract was investigated on HT-29, HeLa, and HEK-293 cell lines according to Alamar blue assay. Harmal methanol extract was found to have near-positive control cytotoxic activity against HeLa cell line and very low cytotoxicity on HT-29 cell line. In addition, harmal methanol extract did not possess any cytotoxicity on HEK-293 cell line. The high amounts of catechin, coumarin, ascorbic acid, and protocatechuic acid were found in harmal methanol extract among 20 phytochemical compounds analyzed. It has been presented that catechin caused apoptosis in HeLa cell line and was highly cytotoxic with an IC_{50} value of 22.91 µg/mL (Rahmaddiansyah et al., 2022). Chuang et al. (2007) demonstrated significant cytotoxic effect of coumarin by causing morphological changes and apoptosis in HeLa cell line with an IC_{50} of 54.2 µM. Ascorbic acid has been shown to have cytotoxicity on HeLa cell line with 202.3 µg/mL IC_{50} value (Abdullah et al., 2021). IC_{50} value of protocatechuic acid was calculated as 40.58 µg/mL on HeLa cell line in the study of Elansary et al. (2020b). This significant cytotoxic activity of harmal can be attributed to the identified phytochemicals mentioned above. Similar to our results, harmal (from Algeria) ethanol extract was recorded as potent cytotoxic active on HeLa cell line with 0.028 mg/mL IC_{50} value. In the same study, harmal seeds aqueous (IC_{50}: 0.230 mg/mL) and decoction (IC_{50}:0.242 mg/mL) extracts were poorly cytotoxic active on HeLa cell line (Mounira et al., 2022). In the study of Khalid et al. (2024), the two different harmal leaves (from Palestinian) ethanol extracts containing 100 µL DMSO and 500 µL DMSO were revealed as low cytotoxic active on HT-29 cell lines with 1350 and 841 mg/mL IC_{50} values, respectively. Elansary et al. (2020a) reported that while cytotoxic activity of harmal leaves methanol extract on HT-29 cell line (IC_{50}: 49.05 µg/mL) was higher than our result, cytotoxic activity on HeLa cell line (IC_{50}: 43.86 µg/mL) was similar to our result. Also, harmal methanol extract was found to be not cytotoxic against HEK-293 cell line (IC_{50}: > 400 µg/mL). The cytotoxicity of harmal seeds (from Palestine) water extract was investigated on COLO205 (IC_{50}: 150.01 µg/mL) and Caco-2 (IC_{50}: 308.35 µg/mL) colorectal cancer cell lines, HeLa cell line (IC_{50}: 155.60 µg/mL), and HEK-293 cell line (IC_{50}: 5.68 µg/mL) by Jaradat et al. (2024). In a different investigation on harmal (from India), isolated compounds harmine, harmaline, vasicinone, and vasicine were found as cytotoxic active on HeLa cell line with IC_{50} values of 61.81, 243.53, 368.57, 335.81 µM, respectively and on HT-29 cell line with IC_{50} values of 45.55, 218.33, 281.24, 291.61 µM, respectively (Ayoob et al., 2017).

5. Conclusion

Through this study, we characterized the phytochemical profile of harmal methanol extract by HPLC and determined the total phenolic and flavonoid amounts. We also focused on antioxidant and cytotoxic activities. Among a total of 20 significant phytochemical compounds, the presence of catechin, coumarin, ascorbic acid, protocatechuic acid, gallic acid, hesperidin, ferulic acid, rutin, and *trans*-cinnamic acid were detected. Harmal methanol extract was found to be rich in total phenolics and flavonoids. Moreover, harmal methanol extract was determined to have valuable antioxidant and cytotoxic (on HeLa cell line) activities comparable to positive controls. Although the results obtained here clearly indicate the possibility of using harmal as a promising antioxidant and cytotoxic agent (especially for cervical cancer), studies on animals and human patients need to be conducted and validated. These results can provide important guidance and can be used as a basis for the development of disease-focused drug discovery. In this context, screening of different bioactive properties of harmal and examination of phytochemicals related to these bioactive properties are needed for further research.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

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