Table 2 —*Pulicaria incisa* aqueous extract cytotoxic and cytostatic effect on HT-29, HeLa and B16–F1 cell lines at different concentrations by LDH assay

	HT-29		HeLa		B16–F1	
Concentration (mg/mL)	Cytotoxic * (%)	Cytostatic (%)	Cytotoxic (%)	Cytostatic	Cytotoxic (%)	Cytostatic (%)
5	98.1	74	63	16.4	71.9	93.1
2.5	87.7	49.1	10.2	11.4	53.6	49.8
1.25	77.3	31.3	8.5	9.3	40.3	37.1
0.6125	64.3	18	2.4	4.8	37.7	34.1
0.3125	61	9.6	0.3	4.4	20.3	31.4

*Percentage of dead cells

Fig. 3 —Percentage of DPPH inhibition activity of the aqueous extracts from *P. incisa* and ascorbic acid.

Total phenol content and antioxidant results

The current research estimated the TPC of *P. incisa* leaf aqueous extract. The TPC was calculated from the regression equation of the calibration curve: ($y = 0.0069x + 0.0696$), $R^2 = 0.9994$, and expressed in gallic acid equivalents per gram dry extract weight. The amount of total phenolic content in this aqueous extract is equal to 155 mg/g in GA equivalent. In addition, the prepared extract showed a high DPPH scavenging power that resembles the scavenging power of ascorbic acid mainly at high concentration, as it reaches around 80% at 50 µg/mL concentration (Fig. 3). Given that, the IC50 value for this aqueous extract was 20.9274 µg/mL.

Discussion

The search for new chemopreventive agents against cancer has shed light on the field of phytochemicals. From the down of the area of medicine, the plant kingdom has been playing a vital role in providing diversity in medications used for cancer treatment[21]. Combination therapy is a smart strategy that enhances the activities of different drugs and reduces both the dose as well as side effects. *P. incisa*, the studied plant species in this research, was reported to be subjected to a different phytochemical screening. The phytochemical analysis of *P. incisa* showed an intermediate amount of total tannins, alkaloids, and saponins reported to have medicinal activity and exhibit physiological activity[22]. High concentrations of carvotanacetone and chrysanthenone were found in both leaves and flower oils[23]. Also, El–Shahaby *et al.* quantified the active secondary ingredients in *P. incisa*, including alkaloids, phenolics, flavonoids, and tannins[24]. Furthermore, *P. incisa* contains large quantities of total flavonoids and total phenolics, especially in the flower[7,25]. Consequently, *P. incisa* total aerial part can be used as an antitumor agent. According to the literature, flavonoids have been shown to have anticancer activities[26]. They intervene with many signal transduction pathways, leading to proliferation, angiogenesis, and metastasis limitation or apoptosis enhancement. Moreover, phenols have antibacterial and anti-inflammatory activities[27]. Also, they are responsible for an antioxidant and free radical scavenging effect of plant materials[28]. In addition to that, phenols possess a wide range of biological activities, most of which are correlated to the control of carcinogenesis and have been identified to affect all stages of cancer progression[29]. In this aspect, phenolic extracts were shown to inhibit the growth of HT-29, and HCT–116 tumour cell line[30]. Likewise, they showed a strong anti-skin cancer effect[31]. Similarly, tannins have been found to have powerful anticancer activities against various cancer cells. Generally, proliferation inhibition, apoptosis induction, invasion suppression, and angiogenesis inhibition are predominantly influenced by tannins[32]. Moreover, several alkaloids isolated from natural herbs have demonstrated both *in vitro* and *in vivo* anti-proliferation and anti-metastasis effects against many types of cancers. Camptothecin and vinblastine are examples of alkaloids that have been successfully formulated to be used as anticancer medicines[33]. In this regard, other compounds such as camptothecin, a topoisomerase I inhibitor, and vinblastine, which interacts with tubulin, have already been successfully

developed into chemotherapeutic drugs[34]. In addition to the mentioned phytochemicals, plant saponins have been already reported to possess a wide range of biological activities[35]. These activities include the anticancer cytotoxic activity of saponins, as they have a significant cytotoxic effect on HeLa cells[36]. Moreover, saponins reveal significant anticancer activities by targeting various cancer-related proteins and pathways, such as cell cycle arrest, apoptosis induction, ER stress activation, migration inhibition, invasion inhibition, and MDR reversal[37]. According to the previous information, the results in the current research indicated that *P. incisa* has significant cytotoxic and cytostatic activity against (Hela), (HT-29), and (B16–F1) cell lines. These activities may be explained by the fact that the prepared extract could be affecting the cell cycle by stimulating cell death through the activation of caspases, in addition to the induction of cell cycle arrest and apoptosis. However, the finding in this research is in accordance with El–Naggar *et al.*, study as they also found that the *P. incisa* extract was one of their tested plants that showed high anticarcinogenic impact on HepG–2 and/or MCF7 cell lines[38]. *P. incisa* provides a high cytotoxic effect against MCF-7 cell lines (less than 30 ug/mL). The presence of secondary metabolites like phenolics and flavonoids that offer antioxidant capacity may be the possible causative agents for such cytotoxic activities. It was shown that the leaf and flower oils of *P. incisa* have cytotoxic activity against liver cell carcinoma HEPG–2 which was determined using an MTT assay. That suggested the efficient cytotoxic activity of leaf oil may be attributed to its high content of carvotanacetone[24]. Also, previous reports have shown that *P. jaubertii* has anticarcinogenic and chemopreventive activity[39,40].

Conclusion

The cytotoxic and cytostatic effect of *P. incisa* against the three cell lines is due to the presence of several phytochemicals. So, it is proposed to use this plant species as a source for antitumor agents either alone or in combination with other agents for cancer treatment. Furthermore, elaborated *in vivo* studies are required to understand the medicinal values against cervical, colorectal, and melanoma cancers. Taken into account, the anticancer effect of plant extracts depends on the plant species, the solvent used, and the used concentrations. In conclusion, traditional medicinal plants in Palestine are a valuable source for the discovery and formulation of new anticancer agents. However, there is a need for further studies that fully characterize their activity in order to exclude or include other compounds that may be found in *P. incisa*.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

1 Qamar H, Rehman S and Chauhan D K, Current status and future perspective for research on medicinal plants with anticancerous activity and minimum cytotoxic value, *Curr Drug Targets*, 2019, **20**(12), 1227-1243.

2 Yuan M, Zhang G, Bai W, Han X, Li C and Bian S, The role of bioactive compounds in natural products extracted from plants in cancer treatment and their mechanisms related to anticancer effects, *Oxid Med Cell Longev*, 2022, **2022**, 1429869.

3 Kowalczyk T, Merecz-Sadowska A, Rijo P, Mori M, Hatziantoniou S, *et al.*, Hidden in Plants—A review of the anticancer potential of the solanaceae family in *In vitro* and *In vivo* studies, *Cancers*, 2022, **14**(6), 1455.

4 Cowan M, Plant products as antimicrobial agents, *Clin Microbial Rev*, 1999, **12**, 564-582.

5 Croteau R, Kutchan T M and Lewis N G, Natural products (secondary metabolites). In *Biochemistry & molecular biology of plants*, B Buchanan, W Gruissem & R L Jones, eds, (American Society of Plant Physiologists, Beltsville, MD, USA), 2000, 1250-1318.

6 Santharam E, Ganesh P, Soranam R, Divya V V, Packia Lekshmi N C J, Evaluation of *in vitro* free radical scavenging potential of various extracts of whole plant of *Calycopteris floribunda* (Lam), *J Chem Pharm Res*, 2015, **7**(1), 860-864.

7 Ewais E, Abd El-Maboud M M and Haggag M I, Studies on chemical constituents and biological activity of *Pulicaria incisa* subsp, Incisa (*Asteraceae*), *Rep Opinion*, 2014, **6**(9), 27-33.

8 Maiza K, *Pharmacopée Traditionnelle Saharienne, Sahara Algérien*, Ph D Thesis, Université Abou Bakr Belkaid, Algerie, 2008.

9 Boumaraf F M, Carbone M, Letizia Ciavatta M, Benyahia S, Ameddah S, *et al.*, Exploring the bioactive terpenoid content of an Algerian plant of the Genus pulicaria, the ent-Series of Asteriscunolides, *J Nat Prod*, 2016, **80**, 82-89.

10 Karim E I, Ishag K E, Elegami A A B, Mahmoud E N and Alfutuh I A, 'D-Carvotanacetone from *Pulicaria undulate*, *Fitoterapia*, 1992, **63**, 281.

11 San Feliciano A, Medarde M, Gordaliza M, Del Olmo E and Del Corral M J M, Sesquiterpenoids and phenolics of *Pulicaria paludosa*, *Phytochem*, 1989, **28**, 2717-2721.

12 Mansour R M A, Ahmed A A, Melek F R and Saleh N A M, The flavonoids of *Pulicaria incisa*, *Fitoterapia*, 1990, **61**(2), 186-187.

13 Ghouila H, Beyaoui A, Jannet H B, Hamdi B, Salah A B, *et al.*, Isolation and structure determination of pulicazine, a new sesquiterpene lactone from the Tunisian *Pulicaria laciniata*, *Tetrahedron Lett*, 2009, **50**, 1563-1565.

14 Feinbrun-Dothan N, *Flora Palaestina*. Text Ericaceae to Compositae, (Israel Academy of Sciences and Humanities), 1977a, 133-145.

15 Feinbrun-Dothan N, *Flora Palaestina*. Plates Ericaceae to Compositae, (Israel Academy of Sciences and Humanities), 1977b, 219-239.

16 Omar G, Abdallah L A, Ismail S and Almasri M Y, Screening of selected medicinal wild plant extracts antibacterial effect as natural alternatives, *Int J Indig Med Plants*, 2013, **46**, 1299-1304.

17 Bopp S K and Lettieri T, Comparison of four different colorimetric and fluorometric cytotoxicity assays in a zebrafish liver cell line, *BMC Pharmacol*, 2008, **8**, 8.

18 Slinkard K and Singleton V L, Total phenol analysis, automation and comparison with manual methods, *Am J Enol Vitic*, 1977, **28**, 49-55.

19 Singleton V L and Rossi J A, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am J Enol Vitic*, 1965, **16**, 144.

20 Abdalah L, Abujafar A, Faisel A, Jawabrah B, Abdullah R, *et al.*, Bioactivity of three medicinal plants leaf aqueous extracts growing wild in Palestine, *Res J Biotechnol*, 2021, **16**(9), 126-134.

21 Lee K H, Anticancer drug design based on plant-derived natural products, *J Biomed Sci*, 1999, **6**, 236-250.

22 Sofowara A, *Medicinal Plants and Traditional Medicine in Africa* (Spectrum Books Ltd, Ibadan, Nigeria), 1993.

23 Shahat E A, Bakr R O, Eldahshan O A and Ayoub N A, Chemical composition and biological activities of the essential oil from leaves and flowers of *Pulicaria incisa* sub, candolleana (Family Asteraceae), *Chem Biodivers*, 2017, **14**(4), e1600156.

24 El-Shahaby O, El-Zayat M, Rabei R and Aldesuquy H S, Phytochemical constituents, antioxidant activity and antimicrobial potential of *Pulicaria incisa* (lam,) DC as a Folk Medicinal Plant, *Prog Chem Biochem Res*, 2019, **2**(4), 222-227.

25 Bakr R O, Shahat E A, Elissawy A E, Fayez A M and Eldahshan O A, Evaluation of the hepatoprotective activity of *Pulicaria incisa* Subspecies *candolleana* and In silico screening of its ısolated phenolics, *J Ethnopharmacol*, 2021, **271**, 113767.

26 Ravishankar D, Rajora A K, Greco F and Osborn H M I, Flavonoids as prospective compounds for anticancer therapy, *Int J Biochem Cell Biol*, 2013, **45**(12), 2821-2831.

27 Vijayvergia R and Kumar J, Quantification of primary metabolites of *Nerium indicum* Mill, *Asian J Exp Sci*, 2007, **21**(1), 123-128.

28 Hasanuzzaman M, Ramjan Ali M, Hossain M, Kuri S and Islam M S, Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits), *Int J Curr Pharm Rev Res*, 2013, **2**(4), 92-96.

29 Dai J and Mumper R J, Plant phenolics, extraction, analysis and their antioxidant and anticancer properties, *Molecules*, 2010, **15**(10), 7313-7352.

30 Zhang Y, Seeram N P, Lee R, Feng L and Heber D, Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties, *J Agric Food Chem*, 2008, **56**(3), 670-675.

31 Wang J J, Shi Q H, Zhang W and Sanderson B J S, Anti-skin cancer properties of phenolic-rich extract from the pericarp of mangosteen (*Garcinia mangostana* Linn,), *Food Chem Toxicol*, 2012, **50**(9), 3004-3013.

32 Cai Y, Zhang J, Chen N G, Shi Z, Qiu J, *et al.*, Recent advances in anticancer activities and drug delivery systems of tannins, *Med Res Rev*, 2016, **37**(4), 665-701.

33 Lu J J, Bao J L, Chen X P, Huang M and Wang Y T, Alkaloids ısolated from natural herbs as the anticancer agents, *Evid Based Complement Alternat Med*, 2012, **2012**, 1-12.

34 Gupta A P, Pandotra P, Kushwaha M, Khan S, Sharma R, *et al.*, Chapter 9- Alkaloids, a source of anticancer agents from nature, *Stud Nat Prod Chem*, 2015, **46**, 341-445.

35 Güçlü-Üstündağ Ö and Mazza G, Saponins, Properties, applications and processing, *Crit Rev Food Sci Nutr*, 2007, **47**(3), 231-258.

36 Khanna V G and Kannabiran K, Anticancer-cytotoxic activity of saponins ısolated from the leaves of *gymnema sylvestre* and *eclipta prostrata* on HeLa cells, *Int J Green Pharm*, 2009, **3**(3), 227-229.

37 Xu X H, Li T, Fong C, Chen X, Chen X J, *et al.*, Saponins from chinese medicines as anticancer agents, *Molecules*, 2016, **21**(10), 1326.

38 El-Naggar S A, Abdel-Farid I A, Elgebaly H A and Germoush M O, Metabolomic profiling, antioxidant capacity and *In vitro* anticancer activity of some compositae plants growing in Saudi Arabia, *Afr J Pharm Pharmacol*, 2015, **9**(30), 764-774.

39 Fawzy G, Al Ati H and El Gamal A, Chemical composition and biological evaluation of essential oils of *Pulicaria jaubertii*, *Pharmacogn Mag*, 2013, **9**(33), 28.

40 Al-Fatimi M, Ali N A A, Wurster M, Al-Sokari S S, Lindequist U and Setzer W N, Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Pulicaria jaubertii* from South Yemen, *World J Pharm Res*, 2015, **4**(12), 1-9.