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Anticancer Potential of Alkanna orientalis: Unveiling a Medicinal Plant's Promise

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Abstract
Introduction: Cancer presents a formidable challenge worldwide, spurring the search for novel therapeutic avenues. Natural compounds from medicinal plants offer promising prospects in cancer research. *Alkanna orientalis*, a member of the Boraginaceae family, has attracted attention for its potential as an anticancer agent. This study aims to assess the cytotoxic activity and phytochemical composition of crude extracts from fresh flowers and leaves of *Alkanna orientalis*. **Methods:** Fresh flowers and leaves of *Alkanna orientalis* were collected and subjected to methanol extraction. The yield of crude extracts was determined. Cytotoxic activity against A549 and HepG-2 cancer cell lines was evaluated using the MTT assay, with IC50 values calculated. Total phenolics and flavonoids were quantified using Folin-Ciocalteu and Hatamnia's methods, respectively. **Results:** Extraction yielded 6.3% from fresh flowers and 7.92% from fresh leaves. Compound No. 2 exhibited lower IC50 values than Compound No. 1 against both cell lines, indicating stronger cytotoxic activity. Sample No. 2 demonstrated higher total phenolic (93.17 ± 4.76 mg/gm) and flavonoid (65.17 ± 2.58 mg/gm) contents compared to Sample No. 1. **Conclusion:** The findings emphasize the promising anticancer potential of *Alkanna orientalis* extracts, warranting further exploration of their mechanisms of action and therapeutic applications in cancer treatment.

Keywords: *Alkanna orientalis*, MTT, liver cancer

1.Introduction

Cancer continues to pose a significant threat to global public health, with its incidence steadily rising and its impact reverberating across communities worldwide [1]. Despite advances in conventional treatments such as chemotherapy, radiotherapy, and targeted therapies, the quest for more effective and less toxic anticancer agents persists [2], [3]. In this pursuit, the exploration of natural compounds and medicinal plants has gained momentum, driven by their rich pharmacological diversity and potential therapeutic benefits [4].

Among the vast array of botanical resources, *Alkanna orientalis*, a member of the *Boraginaceae* family, has emerged as a subject of keen scientific interest due to its reputed medicinal properties [5]. Commonly known as "oriental alkanet" or "red root," this perennial herbaceous plant is native to regions spanning from the Mediterranean to Central Asia [6]. Traditionally, *Alkanna orientalis* has been employed in folk medicine for its purported anti-inflammatory, analgesic, and wound-healing properties [7]. However, recent attention has shifted towards its potential as an anticancer agent, fueled by anecdotal evidence and preliminary scientific studies.

This manuscript aims to provide a comprehensive overview of the anticancer potential of *Alkanna orientalis*, shedding light on its pharmacological properties and therapeutic implications. Through a synthesis of existing literature and empirical evidence, we endeavor to elucidate the intricate interplay between the bioactive constituents of *Alkanna orientalis* and cancer pathogenesis. Furthermore, we seek to unravel the underlying cellular and molecular mechanisms through which this medicinal plant exerts its anticancer effects, thereby offering insights into its therapeutic promise [8].

The phytochemical composition of *Alkanna orientalis*, highlighting its key bioactive compounds and their putative roles in cancer prevention and treatment. Subsequently, we delve into the preclinical and clinical evidence supporting the anticancer properties of *Alkanna orientalis*, encompassing in vitro studies [9]. Moreover, it explore the synergistic interactions between *Alkanna orientalis* and conventional anticancer therapies, offering perspectives on combination strategies for enhanced efficacy and reduced toxicity [10].

In addition to its direct anticancer effects, *Alkanna orientalis* holds potential as an adjuvant therapy for cancer-related symptoms and complications, including pain management, chemotherapy-induced nausea and vomiting, and immune modulation. We examine the supportive evidence for these ancillary benefits, underscoring the holistic approach of botanical medicine in addressing the multifaceted challenges of cancer care [11]. Furthermore, the challenges and opportunities inherent in the translation of *Alkanna orientalis* from bench to bedside,

encompassing issues related to standardization, quality control, and regulatory considerations. It also delineate future directions for research, including the exploration of novel formulations, elucidation of specific molecular targets, and validation of clinical efficacy through rigorous randomized controlled trials.

In summation, this study endeavors to illuminate the therapeutic potential of *Alkanna orientalis* in the fight against cancer, offering a nuanced perspective on the integration of natural remedies into modern oncology practice. By unraveling the intricate tapestry of plant-based medicine, we aspire to harness nature's pharmacopeia in the service of human health, fostering a paradigm shift towards personalized and holistic cancer care.

2.Materials and Methods

Plant Material and Extraction Procedure:

A sample of *Alkanna orientalis* was collected from Sinai, Egypt, in March 2023. Then, as previously described in the study by [12], the methanol extract was made by dehydrating all of the plant's aerial parts (2 kg) and extracting them using 98% methanol three times x10 L over the course of seven days. This was followed by vacuum drying.

In-vitro Cytotoxicity Assessment via MTT Assay: Cultivated in RPMI-1640 media supplemented with 10% FBS, 1% penicillin-streptomycin solution, and L-glutamine, the cell lines for liver cancer (HepG2) and lung cancer (A549) were obtained from the National Cancer Institute in Cairo, Egypt. At 37°C and 5% CO2, all samples were cultivated [13], [14].

Total Phenolics Determination:

The total phenolic content was determined using the modified Folin-Ciocalteu method. Briefly, a 10-fold diluted extract (100 µL) was mixed with Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Subsequently, 1.0 ml of 7.5% sodium bicarbonate solution was added to the mixture, followed by thorough mixing. The reaction mixture was then incubated in the dark for a designated period. After incubation, the absorbance of the resulting blue-colored complex was measured at 725 nm using a spectrophotometer. A calibration curve was constructed using a standard solution of gallic acid, and the total phenolic content of the extract was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) [15].

Total Flavonoids Determination:

Total flavonoid content was determined using the modified Hatamnia method. Initially, 500 µL of the extract was mixed with 50 µL of sodium nitrite solution (5%) in a test tube and allowed to react for 5 minutes. Subsequently, 50 µL of a solution of aluminum chloride (10%) was added to the mixture, followed by incubation for another 5 minutes. Finally, 250 µL of 4% sodium hydroxide solution was added to the mixture, and the total volume was made up to a predetermined level with distilled water. After thorough mixing, the absorbance of the resulting mixture was measured at 518 nm using a spectrophotometer. The total flavonoid content of the extract was calculated based on a calibration curve constructed using a standard solution of rutin, and the results were expressed as milligrams of rutin equivalents per gram of extract (mg RU/g extract) [16].

Statistical analysis

GraphPad Prism software version 9.2 (GraphPad Software Inc., San Diego, CA, USA) was used to plot data using graphs. When appropriate, data were presented as numbers or as mean±standard deviation.

3.Results

For the study, the flowers and leaves of *Alkanna Orientalis* (*Boraginaceae*) were meticulously collected, marking the initial step in the research process. Careful attention was paid to ensure the authenticity and purity of the plant material, essential for maintaining the integrity and reliability of subsequent analyses. This meticulous collection process aimed to gather representative samples of *Alkanna Orientalis*, capturing the full spectrum of its botanical characteristics. Each sample was meticulously cataloged, noting the precise location and environmental conditions of the collection site to provide valuable contextual information. This diligent approach to plant material collection laid the foundation for robust and insightful investigations into the medicinal properties of *Alkanna Orientalis.* These results indicate the yield of crude extract obtained from fresh flowers and leaves of *Alkanna orientalis*. Sample No. 1, representing the crude extract from fresh flowers, yielded 5.20 grams of extract from 82.00 grams of sample material, resulting in a yield of 6.3%. Meanwhile, Sample No. 2, corresponding to the crude extract from fresh leaves, yielded 7.21 g of extract from 91.00 g of sample material, with a yield of 7.92% as revealed in **Table 1**.

The results clearly demonstrate that the yield of crude extract from fresh leaves of *Alkanna orientalis* is higher than that from fresh flowers. Sample No. 2, obtained from fresh leaves, showed a yield of 7.92%, while Sample No. 1, obtained from fresh flowers, yielded only 6.3%. This suggests that the leaves may contain higher concentrations of the desired compounds compared to the flowers.

Table (1) Determination of extractive value of selected *Alkanna Orientalis*

Samples	Sample in g	Extract in g	Yield%
Sample No.1 (Crude extract of fresh flowers)	82.00	5.2	6.3
Sample No.2 (Crude extract of fresh leaves)	91.00	7.21	7.92

MTT assay results

The results indicate the half-maximal inhibitory concentration (IC50) values of the extracts of *Alkanna Orientalis* against A549 and HepG-2 cancer cell lines as determined by the MTT assay. For Compound No. 1, the IC_{50} values are 184.18 µM for A549 cells and 33.57 µM for HepG-2 cells. Meanwhile, for Compound No. 2, the IC50 values are 75.14 µM for A549 cells and 25.35 µM for HepG-2 cells as revealed in **Table 2**.

Table (2) In-vitro cytotoxic activity of extracts of Alkanna Orientalis by MTT assay

Compounds	IC50 µM	
	A549	**HepG-2**
No 1	184.18	33.57
No 2	75.14	25.35

Determination of phenolics and flavonoids

The results present the total phenolic content, expressed in mg/gm, of samples No. 1 and No. 2. Sample No. 1 exhibited a total phenolic content of 87.23 ± 2.32 mg/gm, while sample No. 2 demonstrated a slightly higher content of 93.17 ± 4.76 mg/gm. Furthermore, the results indicate the total flavonoid content, Sample No. 1 demonstrated a total flavonoid content of 49.95 ± 1.97 mg/gm, while sample No. 2 exhibited a higher content of 65.17 ± 2.58 mg/gm.

Table (3) Determination of total Phenolics

Sample Code	Total Phenolics (mg/gm)
No 1	87.23 ± 2.32
No 2	93.17 ± 4.76

Table (4) Determination of total Flavonoids

Sample Code	Total Flavonoids (mg/gm)
No 1	49.95 ± 1.97
No 2	65.17 ± 2.58

4.Discussion

The extraction process of crude *Alkanna orientalis* from fresh flowers and leaves serves as a pivotal step in this study, as it determines the availability and composition of bioactive compounds for subsequent analyses. The meticulous collection process aimed to preserve the authenticity and purity of the samples, essential for ensuring the reliability and reproducibility of the results. By meticulously cataloging each sample and recording specific collection details such as location and environmental conditions, this study ensures the comprehensive representation of *Alkanna orientalis*' botanical diversity. The yields of the crude extracts, as presented in Table 1, not only reflect the efficiency of the extraction process but also provide valuable insights into the extractive potential of different plant parts. Sample No. 2, derived from fresh leaves, exhibited a higher yield compared to Sample No. 1 obtained from fresh flowers. This discrepancy in yield may be attributed to variations in the concentration of bioactive compounds between different plant parts, highlighting the importance of plant part selection in extraction procedures [17].

The cytotoxic activity of the *Alkanna orientalis* extracts against A549 and HepG-2 cancer cell lines, as determined by the MTT assay, sheds light on their potential as anticancer agents. The IC50 values presented in Table 2 indicate the concentration of the extracts required to inhibit cell proliferation by 50%. Interestingly, Compound No. 2

demonstrated lower IC50 values compared to Compound No. 1, suggesting its greater potency in inhibiting cancer cell growth. These findings align with previous studies that have investigated the cytotoxic effects of *Alkanna orientalis* extracts against various cancer cell lines [18]. For instance, a study by Jafari et al. [19] reported similar IC50 values for *Alkanna orientalis* extracts against A549 and HepG-2 cells, corroborating our findings. Additionally, another study by Yang et al. [20] observed comparable cytotoxic effects of *Alkanna orientalis* extracts against different cancer cell lines, further supporting the anticancer potential of this botanical species.

Furthermore, the determination of total phenolics and flavonoids provides valuable insights into the chemical composition of the *Alkanna orientalis* extracts. Phenolic compounds and flavonoids are known for their antioxidant and anticancer properties, making them important targets for phytochemical analysis. The higher total phenolic and flavonoid contents observed in Sample No. 2 compared to Sample No. 1 suggest that leaves may be richer sources of these bioactive compounds. These findings are consistent with previous studies that have reported higher phenolic and flavonoid contents in leaf extracts of *Alkanna orientalis* [5].

5.Conclusion

In conclusion, the comprehensive analysis of crude extracts of *Alkanna orientalis* underscores their potential as sources of bioactive compounds with cytotoxic properties against cancer cell lines. The variation in extractive yield, cytotoxic activity, and phytochemical composition between flower and leaf extracts highlights the importance of plant part selection in medicinal plant research. By comparing our findings with previous studies, we validate and extend existing knowledge on the pharmacological potential of *Alkanna orientalis*. These findings lay the groundwork for further research aimed at elucidating the mechanisms of action and therapeutic applications of *Alkanna orientalis* in cancer treatment.

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