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ASSESSING THE *IN VITRO* ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF *ALPINIA OFFICINARUM* AND *HUMULUS LUPULUS* EXTRACTS

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ABSTRACT

In this study, we investigated the cytotoxic and antioxidant properties of acetone extracts obtained from *Alpinia officinarum* (*Al. officinarum*) and *Humulus lupulus* (*H. lupulus*). The assessment encompassed the computation of the extracts' total flavonoid content (TFC) and total phenolic content (TPC), revealing noteworthy concentrations of both phenolic and flavonoid components. According to DPPH and FRAP tests, both extracts had high antioxidant activity. Their low IC_{50} values suggested that they were capable of scavenging free radicals. Several bioactive substances were detected in each extract by gas chromatography-mass spectrometry (GC-MS) analysis. These chemicals included ç-Sitosterol and n-Hexadecanoic acid in *Al. officinarum* and vitamin E and α-amyrin in *H. lupulus*. These results point to the plant extracts' potential for therapeutic use. Furthermore, assessments of the cytotoxicity of hepatocellular carcinoma (HepG2), lung cancer (A549), and breast cancer cell lines (MCF-7). The *Al. officinarum* extract shown strong cytotoxicity against each of the three cell lines, with IC_{50} values for each cell line ranging from 107.10 µg/mL to 51.57 µg/mL and greater than 100 µg/mL. *H. lupulus* extract exhibited cytotoxic properties as well, with IC_{50} values greater than 100 µg/mL. These results highlight the potential of extracts from *Al. officinarum* and *H. lupulus* as sources of bioactive components with cytotoxic and antioxidant qualities, indicating their potential use in the creation of innovative medications. The significance of medicinal plants in the search for novel therapeutic molecules is highlighted by this study.

Key words: : *Al. officinarum, H. lupulus***,** GC-MS, HepG2**,** A549**,** MCF-7

INTRODUCTION

According to **Rodrigues *et al.* (2016)**, natural products, especially those sourced from plants; offer a promising source of new biologically active substances. Due in large part to the harmful effects of synthetic drugs, herbal products have grown increasingly popular **(Bahadori *et al.*, 2019).** Natural resources have promise as antioxidants and anticancer agents due to their various secondary metabolites and rich polyphenolic contents. By stopping the creation of free radicals or neutralizing them after they are produced, antioxidants can successfully postpone or prevent oxidative damage, which is the underlying cause of many diseases. There is a growing interest in finding useful natural components for human usage because natural chemicals are generally more reliable and effective than manufactured antioxidants (**Al-Snafi, 2016**).

Medicinal herbs have been the main source of natural antioxidants for treating human illnesses **(Prathapan *et al.*, 2011).** *Alpinia officinarum* is a perennial herb in the Zingiberaceae family that is predominantly grown in Southeast Asia, and it originated in China (**Anonymous 2004; Zhang *et al.*, 2010a**). This herb is also grown in the Eastern Himalayan plains of West Bengal and Assam. Reaching a height of 10 feet or more, it features lanceolate leaves and reddish-white blooms. The slender, vigorous rhizomes of this herb are called galangal, and their orange meat is covered in a brown covering that tastes hot and pungent and has a pleasant aroma (**Gupta and Tendon 2004a, Gupta and Tendon 2004b, Sun *et al.*, 2008**). Numerous phytochemicals, such as quercetin, kaempferol, isorhamnetin, kaempferide, galangin, alpinol, and galangol, have been found in the herb (**Deng *et al.*, 2011; Yang *et al.*, 2011**). *Alpinia officinarum* has a long history of traditional use and is highly valued for its wide range of therapeutic benefits. Significant anti-inflammatory, antibacterial, antifungal, antiviral, diuretic, and anticancer properties have been reported by research (**Lee *et al.*, 2009; Konno *et al.*, 2011**).

Within the Cannabaceae family, common hop (*Humulus lupulus*) is a perennial climbing plant that grows in temperate northern climes. Numerous physiologically active substances with strong antibacterial, antioxidant, and antifungal effects may be found in all of its sections (**Bocquet *et al.,* 2019**; **Abram *et al.*, 2015**). When it was first used for medical purposes, it was eventually used mostly in the brewing of beer. Hops have new and exciting prospects outside of the beer business because of these plant properties and the growing interest in biologically active compounds that promote health (**Abram *et al.*, 2015; Bocquet *et al.*, 2019**). From ages ago to the present, hops have been cultivated for a variety of items, such as tea, fishing gear (which uses the stems), food products (which cook with the young shoots), and even bread preservative (**Abram *et al.*, 2015; Nionelli *et al.*, 2018**). Even though hop qualities have been studied extensively over the past 25 years (**Karabín *et al.*, 2016**), further research is required to clarify the chemical makeup of hop leaves and establish whether they are equivalent to cones in terms of bioactive

chemicals. Furthermore, research is required to see whether other strategies may be used to improve hops' ability to synthesize beneficial chemicals. Thus, the objective of the study was to investigate the *in vitro* antioxidant and cytotoxic properties of acetone extracts of *Alpinia officinarum* (*Al. officinarum*) and *Humulus lupulus* (*H. lupulus*).

MATERIALS AND METHODS

The *Al. officinarum* and *H. lupulus* plants were sourced from the Faculty of Agriculture, Minia University plant nursery. Fresh and healthy leaves were carefully cleaned, properly cleaned under running tap water, and allowed to air dry for four weeks away from direct sunlight in a typical atmosphere. After that, the leaves were placed in a sealed container after being finely processed into a powder using an electric grinder. 100 grams of the powdered material were added to a 1000 mL round-bottom flask equipped with a magnetic stirrer in order to prepare the extract. For six hours, the mixture was constantly stirred after 700 mL of acetone was gradually added to the flask. The final extract was collected and filtered through Whatman No. 1 filter paper. The filtrate was evaporated in a rotary evaporator operating under vacuum at 45°C until a residue developed. After that, this residue was kept at -20°C until further use.

Total phenolic content (TPC), Total Flavonoids Content (TFC) and Antioxidant activity of extracts:

TPC for every extract was assessed using the Folin-Ciocalteu technique, which is explained by **Velioglu *et al.* (1998)** and **Singleton *et al.* (1991).** The phenolic content was measured in milligrams of gallic acid equivalent (mg GAE) per gram of the sample. Furthermore, the method described by **Ebrahimzadeh *et al.* (2008)** was used to determine the extracts' total flavonoid content. The TFC content was measured in milligrams of quercetin equivalents (mg QE) per gram of the sample.

The evaluation of antioxidant activity was performed using the '2,2'- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging technique. Extracts from different plant samples were tested for their capacity to scavenge free radicals using the method outlined by **Brand-Williams *et al.* (1995).**

The protocol described by **Benzie and Strain (1996)** was adhered to for performing the Ferric Reducing Antioxidant Power (FRAP) test.

GC-MS Analysis:

The National Research Center's Mass Spectrum Lab in Dokki, Giza, is where the GC/MS analysis took place. This investigation made use of a TG-5MS fused silica capillary column (30 m length, 0.25 mm diameter, and 0.1 mm film thickness) and a Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS. Helium gas was used as the carrier in an electron ionization system operating at 70 eV of ionization energy, maintaining a flow rate of 1 mL per minute for GC/MS detection. The injector and MS transfer line were continuously maintained at 280°C. All components that were found were quantified using the percent relative peak area method. Comparing the mass spectra and relative retention periods of the compounds to the NIST and WILLY

library data allowed for a preliminary identification of the compounds (**Joulain and Konig, 1998).**

Cell culture and reagents

Cells for hepatocellular carcinoma (HepG2), lung cancer (A549), and breast cancer (MCF-7) were obtained from Nawah Scientific Inc. in Mokattam, Cairo, Egypt. The medium used to cultivate and maintain these cells was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 mg/mL streptomycin, and 100 units/mL penicillin. These cell lines were maintained at 37°C in a humidified atmosphere containing 5% (v/v) carbon dioxide.

Cytotoxicity assay

The sulforhodamine B (SRB) assay was used to evaluate the vitality of the cells. Initially, 96-well plates were seeded with 100 µL of a cell suspension containing 5x10 ^3 cells, and the plates were incubated in complete medium for a full day. Subsequently, 100 µL of medium with varying amounts of plant extracts were introduced to the cells. Fixation was performed by replacing the medium with 150 µL of 10% TCA after 72 hours of exposure, and then incubating the mixture for 1 hour at 4°C. The cells were rinsed five times with distilled water after the TCA was removed. In order to assess viability, 70 µL of SRB solution (0.4% w/v) was added, and it was incubated for 10 minutes at room temperature in a dark setting. The plates were then allowed to air-dry for an entire night after being cleaned three times using 1% acetic vinegar. Afterwards, 150 µL of TRIS (10 mM) was added to dissolve the SRB stain that was attached to the protein. A BMG LABTECH® FLUOstar Omega microplate reader was then used to measure the absorbance at 540 nm. (**Skehan *et al*., 1990; Allam *et al*., 2018**).

Statistical analysis:

The analyses and experiments were run three times, and the results are presented as means with standard deviations (mean ± SD). The significance of the treatment effect was determined using the F-test ($p < 0.05$). **Duncan's test (1957)** was used to assess differences between individual means, with a significance threshold of $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis and antioxidant activity

Phenolics, also known as polyphenols, are important secondary chemicals found in plants because of their antioxidant properties. Their mode of action consists of attaching to metal ions that are redox active, deactivating chains of free radicals in lipids, and preventing hydroperoxides from becoming reactive oxyradicals. They can demonstrate their antioxidative qualities thanks to this technique. Using spectrophotometric methods, TPC and TFC contents of the acetone extracts of *Alpinia officinarum* and *Humulus lupulus* were investigated. The results are presented in Table 1. Based on the data obtained, the total phenolic content was found to be 37.56±0.09 mg GAEs/g for *Al. officinarum* extract and 40.03±0.018 mg GAEs/g for *H. lupulus* extract.

Additionally, the total flavonoid content was measured at 27.08±0.41mg QEs/g for *Al. officinarum* extract and 29.61±0.46 mg QEs/g for *H. lupulus* extract. **Srividya *et al.* (2010)** conducted a qualitative phytochemical analysis of the *Alpinia officinarum* rhizome extract, revealing the presence of several compounds predominantly tannins, alkaloids, flavonoids, and saponins. Moreover, the hydroalcoholic extract, prepared through a hot maceration process, exhibited higher concentrations of phenols and flavonols, measured at 50.1 mg/g and 54.02 mg/g, respectively.

In their study, **Wang *et al.* (2014)** examined the phenolic makeup of a hop polyphenol extract that was highly pure and had an exceptionally high total phenolic content of 887 mg/g. It has been observed that the polyphenol content of hops (*Humulus lupulus* L.) usually falls between 40 and 140 mg/g.

Antioxidants are essential for preventing or lessening the harmful effects of reactive oxygen species by transforming them into innocuous molecules. According to **AL-Dabbas (2017),** this preventive effect covers a range of medical disorders, such as cancer, heart disease, infections, diabetes, and ischemia. A single technique might not adequately capture the antioxidant potential of bioactive secondary metabolites in plant extracts due to their complex composition (**Du *et al.*, 2009**). Thus, two main techniques were mostly used to evaluate the antioxidant activity of *Al. officinarum* and *H. lupulus* extracts. Table 1 displays the outcomes of various techniques, particularly the FRAP and DPPH free radical scavenging assays.

Table 1 offers a visual representation of the ability of *Al. officinarum* and *H. lupulus* extracts to scavenge DPPH free radicals. For both the *Al. officinarum* and *H. lupulus* extracts, the IC_{50} value—which stands for the concentration at which 50% of the radicals are neutralized—was determined to be 36.52µg/ml and 36.58µg/ml, respectively. By suppressing DPPH free radicals, the hydroalcoholic extract made by a heat maceration procedure showed a concentration-dependent ability to scavenge radicals, according to **Srividya *et al.* (2010).** Even while the three extracts showed some modest reducing power, ascorbic acid, the conventional antioxidant, was much more efficient than the other two. Among the extracts, the hydroalcoholic extract that was produced by hot maceration was particularly noteworthy for having higher antioxidant activity. Additionally, the scientists proposed that the existence of phenolic components, such as flavonoids and phenolic acids. With determined values of 115.69±4.31 mg TEs/g for *Al. officinarum* extracts and 667.79±18.70 mg TEs/g for *H. lupulus* extracts, both extracts showed considerable FRAP activity.

According to **Önder *et al.* (2013),** the n-hexane extract obtained from *H. lupulus* showed the highest antioxidant activity against DPPH, measured at 14.95 ± 0.03 µg Trolox equivalent/g sample.

Zhang *et al.* (2017) found that the *Al. officinarum* extract had a phenolic content value of 5.20±0.14 GAE mg g^{-1}, a flavonoid content of 14.38±1.67 QE mg g^{-1}, and a DPPH of 190.11±0.36 (Ascorbate equivalent (µM).

Table 1: Total phenolic content (TPC), total flavonoid content (TFC) and Antioxidant activities of *Al. officinarum* and *H. lupulus* extracts

	Al. officinarum	*H. lupulus*
Total Flavonoids (mg QE/g extract)	27.08±0.41	29.61±0.46
Total Phenolic ((mg GAE/g extract)	37.56±0.09	40.03±0.018
DPPH IC_{50}(μg/ml)	36.52	36.58
FRAP (μM TE /mg)	115.69±4.31	667.79±18.70

Values are mean ± SD (standard deviation).

Medicinal plants are unquestionably a priceless source for the development of new drugs because they have been instrumental in the identification of active ingredients used in modern medical therapies. Using plant-based reservoirs has made it easier to identify a variety of bioactive compounds that are essential for treating a broad range of illnesses. The crucial steps in modernizing and ensuring the quality of herbal formulations are closely examining and separating these chemicals from plant materials (**Rathor, 2021**).

The bioactive chemicals included in the acetone extracts obtained from *Al. officinarum* and *H. lupulus* were identified using the GC-MS method. Detailed information concerning the chemicals detected in the extracts of *Al. officinarum* and *H. lupulus* may be found in Tables 2 and 3. It is crucial to highlight that the findings of the GC-MS analysis for the remaining extracts, which displayed either low or no cytotoxic activity, are not included in this analysis. The results of this study, which are presented in Table 2 and Fig 1, demonstrate that the acetone extract of *Al. officinarum* contains 17 bioactive phytochemical components. Prominent components found in *Al. officinarum*'s acetone extract include Hexadecanoic acid trimethylsilyl ester (18.70%), Oleic Acid, (Z)-, TMS derivative (10.30%), Oleic Acid (8.48%), and Bicyclo[2.2.2].Oct-5-Ene, 2-Methyl, 2-Methylic Acid, Dimethyl Ester, (1à, 2á, 3à, 4à)-(6.01%).

As shown in Table 3 and Fig 2, the acetone extract obtained from *H. lupulus* revealed the existence of 19 bioactive chemicals using GC-MS analysis. Prominent bioactive components, such as ç-Sitosterol (23.31%), n-Hexadecanoic acid (19.13%), α-Amyrin (12.04%), 9-Octadecenoic acid, (E)- (6.76%), 9,12-Octadecadienoic acid (Z,Z)- (5.16%), and Betulinaldehyde (4.51%), were found in noteworthy proportions in the acetone extract.

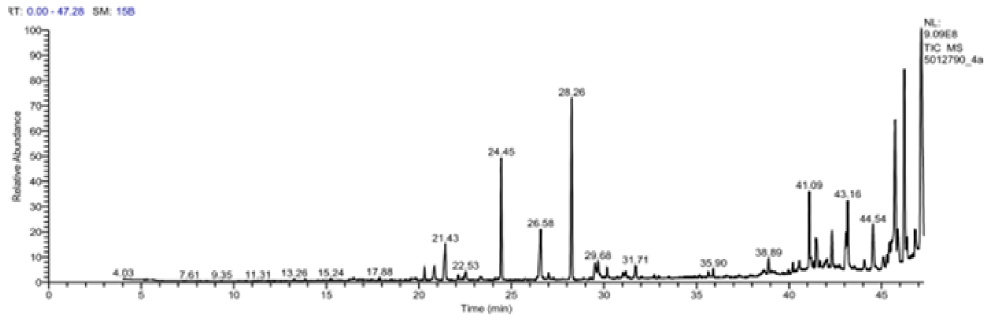

Fig.1: GC-MS chromatogram of acetone extract of *Al. officinarum*

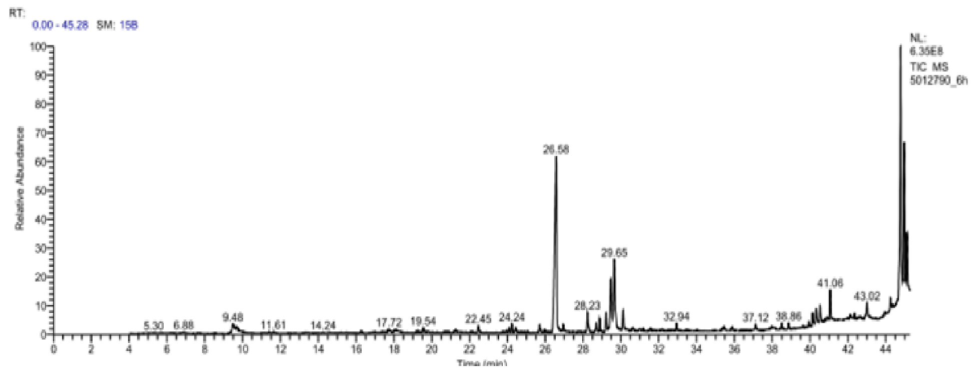

Fig.2: GC-MS chromatogram of acetone extract of *H. lupulus*

Table 2: Identification of Phytochemical Components in Acetone Extract of *Al. officinarum* by GC-MS analysis.

No.	Compound name	Molecular formula	Area %	MW	RT
1	Pheniramine	$C_{16}H_{20}N_2$	1.14	240	14.59
2	4-Hydroxybenzaldehyde, TMS Derivative	$C_{10}H_{14}O_2Si$	1.24	194	13.18
3	ç-Sitosterol	$C_{29}H_{50}O$	1.38	414	45.40
4	Trimethylsilyl (9e)-9-Octadecenoate	$C_{19}H_{34}O_2$	1.42	354	37.49
5	Silane, trimethyl.(stigmast.-5.-en-3β-yloxy.)-, (24S)-	$C_{32}H_{58}OSi$	1.48	486	46.23
6	(S)-4-(1-Acetoxyallyl)phenyl acetate	$C_{13}H_{14}O_4$	1.62	234	19.29
7	Octadecanoic Acid	$C_{18}H_{36}O_2$	2.09	284	30.25
8	Aucubin, hexakis (trimethylsilyl) Ether	$C_{33}H_{70}O_9 Si_6$	2.17	778	46.67
9	Stearic acid, TMS derivative	$C_{21}H_{44}O_2 Si$	2.51	356	31.69
10	Linoelaidic acid, trimethylsilyl ester	$C_{21}H_{40}O_2 Si$	2.70	352	31.04
11	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	3.28	328	28.23
12	Hexadecanoic Acid	$C_{16}H_{32}O_2$	3.50	256	26.56
13	1,2,2-(2h(3)-4-Methoxypheny Lethene	$C_9H_7D_3O_2$	4.07	137	37.93
14	Bicyclo[2.2.2.]Oct-5-Ene.-2,3-Dicarboxylic Acid, 2-Methyl-., Dimethyl Ester,(1à.,2á,3à,4à.)-	$C_{13}H_{18}O_4$	6.01	328	31.59
15	Oleic Acid	$C_{18}H_{34}O_2$	8.48	282	29.84
16	Oleic Acid, (Z)-, TMS derivative	$C_{21}H_{42}O_2Si$	10.30	354	31.20
17	Hexadecanoic Acid, Trimethylsilyl Ester	$C_{19}H_{40}O_2 Si$	18.70	328	28.29

Table 3: Identification of Phytochemical Components in Acetone Extract of *H. lupulus* by GC-MS analysis

No	Compound name	Molecular formula	Area %	MW	RT
1	Tetradecanoic acid	$C_{14}H_28O_2$	0.63	228	22.45
2	4,8,12,16-Tetramethylheptadecan-4-solide	$C_{15}H_{24}$	0.64	204	41.45
3	2-Hexadecen-1-ol,3,7,11,15.-tetramethyl.-,[r.[r*,r*-(e)]]-	$C_{20}H_{40}O$	0.72	296	24.24
4	Hexadecanoic Acid, Methyl Ester	$C_{31}H_{50}O_3$	0.74	270	25.69
5	8,11-Octadecadienoic Acid, Methyl Ester	$C_{19}H_{34}O_2$	0.86	294	28.70
6	Brenzkatechin	$C_6H_6O_2$	0.95	110	9.47
7	1-Heptatriacotanol	$C_{37}H_{76}O$	1.13	536	44.25
8	10-Octadecenoic Acid, Methyl Ester	$C_{19}H_{36}O_2$	1.21	296	28.88
9	Phytol	$C_{20}H_{40}O$	1.43	296	29.21
10	Dimethoxycurcumin	$C_{23}H_{24}O_6$	1.55	396	40.32
11	Vitamin E	$C_{29}H_{50}O_2$	1.56	430	43.01
12	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	1.84	328	28.23
13	Heptacosane	$C_{27}H_{56}$	2.46	380	41.06
14	Betulinaldehyde	$C_{30}H_{50}O_2$	4.51	440	45.12
15	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	5.16	280	29.47
16	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	6.76	282	29.65
17	ɑ –Amyrin	$C_{30}H_{50}O$	12.04	426	44.98
18	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	19.13	256	26.58
19	ҫ-Sitosterol	$C_{29}H_{50}O$	23.31	414	44.79

Cytotoxic activity

In order to aid in the development of possible antiproliferative drugs, the cytotoxicity of extracts from *Alpinia officinarum* and *Humulus lupulus* was assessed on three different cell lines: Hepatocellular carcinoma (HepG2), Lung cancer (A549), and breast cancer (MCF-7) cells. Figures 3-5 describe how the SRB assay was used to measure cell viability and compare it to untreated cells that were used as a control. The percentage of viable cells after exposure to several doses of extracts from *Al. officinarum* and *H. lupulus* is shown in Fig 6**.**

Al. officinarum extract exhibited significant cytotoxic effects against A549, HepG2, and MCF-7 cell lines, with IC_{50} values of 51.57 μg/mL, 107.10 μg/mL, and >100 μg/mL, respectively. The results show that *Al. officinarum* extract showed moderate antiproliferative activity against the A549 cancer cell, as shown in Table 4. In contrast, the IC_{50} values of *H. lupulus* extract for A549, MCF-7, and HepG2 cell lines exceeded 100 μg/mL. The relative viability of the three tested human tumor cell lines (A549, HepG2, and MCF-7) decreased with an increase in the concentrations of *Al. officinarum* and *H. lupulus*, as illustrated in Fig. 6.

The presence of bioactive chemicals is probably responsible for the cytotoxic activities that have been observed. Natural products are widely acknowledged as prospective sources for new therapeutic medicines (**Masood *et al.*, 2011; Javed and Qadir, 2011**). The MCF-7 cell viability (IC_{50}: 43.45 μg/mL for 48 hours) and the LNCaP cell viability (IC_{50}: 168 μg/mL for 48 hours) were both markedly decreased by the hydroalcoholic extract. But at 200 and 400 μg/mL (72 hours), the aqueous extract only decreased cancer cell viability by more than 50%. These extracts (25–100 μg/mL; 24 and 48 hours) did not significantly reduce the viability of primary fibroblasts. **Kazemi *et al.* (2022)** reported that the hydroalcoholic extract significantly increased the number of apoptotic cells in both MCF-7 and LNCaP cells.

Additionally, enantiomers of a β, β-unsaturated ketone (2) and β-hydroxyketone (1) of the diarylheptanoids derived from *Al. officinarum* have been synthesized effectively using commercially available eugenol. Studies by **Gamre *et al.* (2021)** revealed that compound (2) among these compounds had more effective antiproliferative activity against human breast cancer MCF-7 cells. Hop seed extract's cytotoxicity against multiple cancer cell lines, including MCF-7 (breast adenocarcinoma), HeLa (cervical carcinoma), NCI-H460 (non-small cell lung cancer), and HepG2 (hepatocellular carcinoma), is demonstrated by its aqueous extract, which exhibits cytotoxicity against each of these lines with IC_{50} values of ≤ 278 ± 4 μg/mL. An extract concentration of 184 ± 6 μg/mL inhibited 50% of the growth of NCI-H460 tumor cells, which was an especially significant effect. **Alonso-Esteban *et al.* (2019)** have established a possible correlation between the high amount of catechin in the extract and the reported cytotoxic effects.

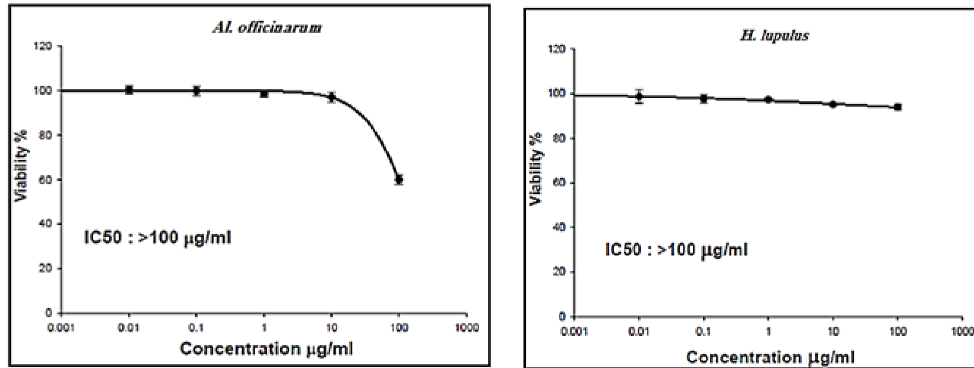

Fig 3. Cytotoxic activity of *Al. officinarum* and *H. lupulus* extracts against (MCF-7)

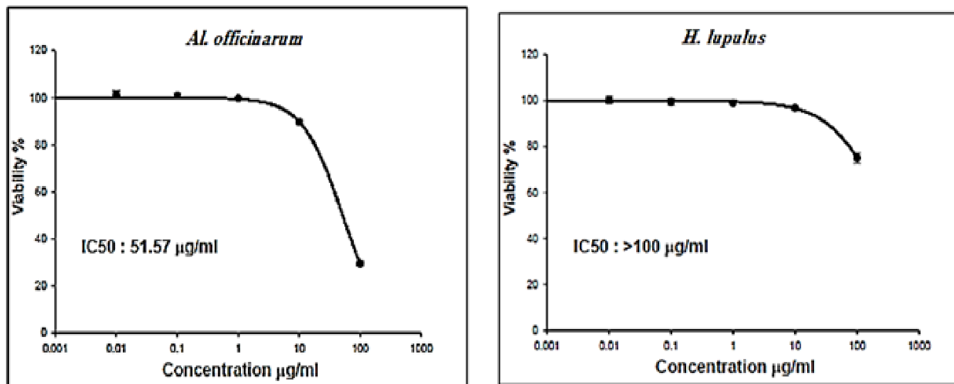

Fig 4. Cytotoxic activity of *Al. officinarum* and *H. lupulus* extracts against (A549)

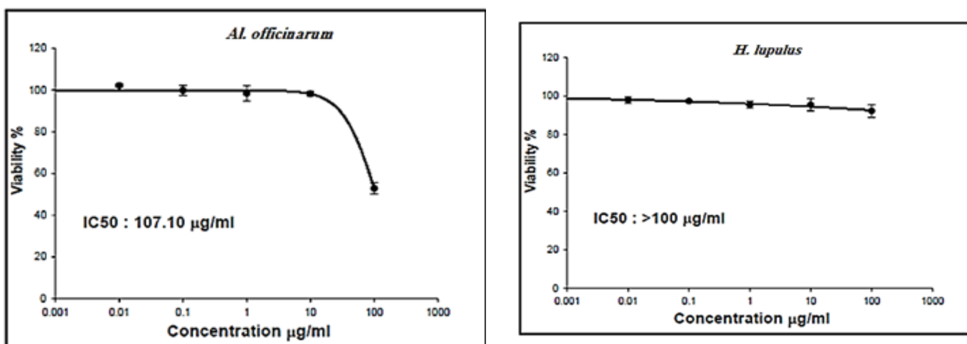

Fig 5. Cytotoxic activity of *Al. officinarum* and *H. lupulus* extracts against (HepG2)

Table 4: Cytotoxic activity of *Al. officinarum* and *H. lupulus* extracts against MCF-7, A549, and HepG2 cell lines.

	IC_{50} (µg/ml)	
	Al. officinar	*H. lupulus*
MCF-7	>100	>100
A549	51.57	>100
HepG2	107.1	>100

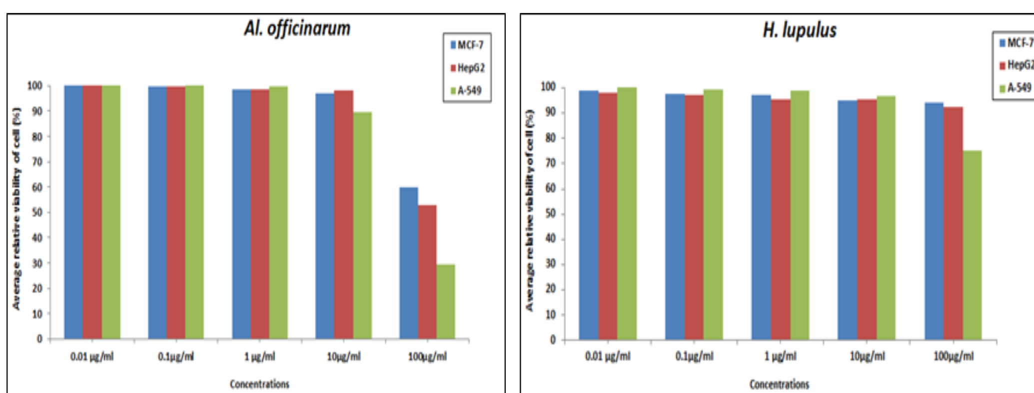

Fig 6. Mean Relative Viability of *Al. officinarum* and *H. lupulus* extracts against MCF-7, A549, and HepG2 cell lines cell line.

Conclusion

In conclusion, the study demonstrated the antioxidant potential of *Al. officinarum* and *H. lupulus* extracts by showing that they both contain sizable concentrations of phenolic and flavonoid components. These extracts displayed considerable antioxidant activity in DPPH and FRAP experiments, with IC_{50} values showing their efficacy in scavenging free radicals. Multiple bioactive chemicals were found in both extracts by GC-MS analysis, with *Al. officinarum* and *H. lupulus* exhibiting potential therapeutic use. Furthermore, the extracts' effectiveness against several cancer cell lines was shown by cytotoxicity experiments. These results emphasize the importance of these plant extracts as stores of bioactive substances with cytotoxic and antioxidant properties. This illustrates the possibility of developing innovative medications in the future.

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الملخص العربي

تقييم الخواص المضادة للأكسدة والسمية الخلوية في مستخلصات الخولنجان وحشيشة الدينار في المختبر

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في هذه الدراسة، قمنا بدراسة الخصائص السامة للخلايا ومضادات الأكسدة لمستخلصات الأسيتون التي تم الحصول عليها من حشيشة الدينار (*Al. officinarum*) *Alpinia officinarum* والخولنجان *Humulus lupulus* . (*H. lupulus*)وشمل التقييم حساب محتوى الفلافونويد الإجمالي للمستخلصات (TFC) والمحتوى الفينولي الإجمالي (TPC)، مما يكشف عن تركيزات جديرة بالملاحظة لكل من المكونات الفينولية والفلافونويد. وفقا لاختبارات DPPH وFRAP، كان لكلا المستخلصين نشاطا مرتفعا مضادا للأكسدة. تشير قيم IC_{50} المنخفضة الخاصة بها إلى أنها قادرة على التخلص من الجذور الحرة. تم الكشف عن العديد من المواد النشطة بيولوجيا في كل مستخلص عن طريق التحليل اللوني للغاز ومطياف الكتلة .(GC-MS) وشملت هذه المواد الكيميائية ç-Sitosterol n-Hexadecanoic acid في *Al. officinarum* وفيتامين E و α-amyrin في*H. lupulus*. وتشير هذه النتائج إلى إمكانية استخدام المستخلصات النباتية علاجيًا. وعلاوة على ذلك، تم إجراء تقييمات السمية الخلوية لخطوط خلايا سرطان الكبد(HepG2) ، وسرطان الرئة(A549) ، وسرطان الثدي(MCF-7) . أظهر مستخلص *Al* officinarum سمية خلوية قوية ضد كل خط من خطوط الخلايا السرطانية الثلاثة، حيث تتراوح قيم IC_{50} لكل خط خلية من 107.10 ميكروجرام/مل إلى 51.57 ميكروجرام/مل وأكثر من 100 ميكروجرام/مل. أظهر مستخلص *H. lupulus* خصائص سامة للخلايا أيضًا، مع قيم IC_{50}أكبر من 100 ميكروغرام/مل. هذه النتائج تسلط الضوء على إمكانات مستخلصات من *Al.* officinarum و *H. lupulus* كمصادر للمكونات النشطة بيولوجيًا ذات الصفات السامة للخلايا ومضادات الأكسدة، مما يشير إلى إمكانية استخدامها في تحضير أدوية مبتكرة. كما تسلط هذه الدراسة الضوء على أهمية النباتات الطبية في البحث عن جزيئات علاجية جديدة.