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Investigating the Effect of Alcoholic Extract as Antimicrobial, Antioxidant, and Anticancer Drug Biosynthesized from Onion Allium Plant

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ABSTRACT

Background and Objective: The use of chemical drugs results in substantial adverse effects and the development of drug resistance. *Onion Allium cepa* (*O. Allium cepa*) is one of the most well-known herbal treatments due to its ability to reduce inflammation and strengthen the body's immune system. The aim of this study is to investigate the effect of alcoholic extract as an antimicrobial, antioxidant and anticancer drug biosynthesized from *Onion Allium*.

Methods: In this laboratory study, the ethanolic extract of the *onion Allium cepa* plant was used to prepare the biosynthesis using a soxhlet with 75% ethanol. The UV/Vis spectrophotometry (UV-vis) and Fourier transmission infrared analysis (FTIR) were employed for characterization of ethanolic extract. Antibacterial activities of ethanolic extract against *Escherichia coli (E. coli)* and *Staphylococcus aurous (S. aurous)* were investigated. The cytotoxic effect of alcoholic extract on AMJ-13 breast cancer cell line at different concentrations (6.125, 12.5, 25, 50 and 100) micrograms/ml was evaluated by MTT method and DPPH was used to evaluate the antioxidant activity.

Findings: According to the results of Fourier transmission infrared analysis and phytochemical screening, numerous active substances, including polysaccharides, saponins, phenolic compounds, glycosides, and flavonoids were found in this extract. The antimicrobial activity test results revealed the largest inhibition zone in *Escherichia coli* as compared to *Staphylococcus aureus*. The AMJ-13 cell line's proliferation was greatly reduced by this extract in a concentration-dependent way when compared to the control, whereas this extract's cytotoxic activity increased with concentration. The findings demonstrated that *Onion Allium cepa* is a principally valuable source of powerful anti-oxidant.

Conclusion: According to the results of this study, ethanolic extract of the *onion Allium cepa* plant has antibacterial activity and reduced malignant cell lines' ability to proliferate and showed a strong antioxidant impact.

Keywords: *Onion Allium, Fourier Transmission Infrared Analysis, Biology Activity*.

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Introduction

The onion, *Allium cepa*, is a perennial herbaceous plant that grows from rhizomes, bulbs, swollen fleshy roots, or tubers. It is a member of the Liliaceae family. Its lower surface is made up of fibrous adventitious roots, and its upper surface is made up of fat leaves that serve as the base of the leaves. Its cover surrounds the bulb, which is the part that is used for medicine (1). Onions have many medical benefits, and they are used in the treatment of arthritis and rheumatism, and treatment of skin diseases (2). The efficacy of alcoholic onion extract has been proven in the treatment of skin allergies and bronchial obstruction in guinea pigs and rabbits, as it has an effect similar to that of histamine (3). The concentrated aqueous extract of this plant is used for people with diabetes (4), and onions have efficacy in treatment of cancerous diseases (5). The researchers showed that the essential oils of onion and garlic have anti-growth effects on different types of bacteria and fungi (6). Santas et al. confirmed that a number of onion extracts have an effect on some types of bacteria isolated from the tonsils (7). Sharma et al. confirmed that onion extracts have an effect on pathogenic bacteria in the mouth (8). There are many anti-microbial compounds that cost a lot and many of them have side effects, in addition to the fact that the continuous use of them in treatment loses effectiveness and bacteria gain resistance to it. Thus, a group of countries paid great attention towards medicinal plants as a natural source of medicines, and there have been many attempts in recent years to investigate the effect of a number of medicinal plants on microorganisms isolated from patients with different diseases (9, 10). The onion plant is highly important in folk medicine, and is rich with compounds that combat the growth of bacteria.

This study shed light on one of the aspects among enormous natural wealth of Iraq's environment, which aims to prepare extracts and separate a number of active ingredients from onions, and evaluate the effectiveness and antibacterial activity of the extract against two types of bacteria, in addition to antioxidant, anticancer activity.

Methods

Preparation of Ethanolic Extract: Using soxhlet and 75% ethanol, the dried *Onion allium* plant was extracted. The extracts had been entirely eliminated by using a rotating evaporator, and a semi-solid mass was gained by an oven that was used for drying, and was stored at 4°C until needed (11).

Screening of ethanolic extract for chemical compounds: The crude extracts' phytochemical components, including saponins, phenolic, reducing sugar, steroids, glycosides, and flavonoids, were examined. In each analysis, two milliliters of onion allium plant extract were used in such a way that the appearance of a uniform precipitate, a change in color, or bubbling indicated that it contained the mentioned phytochemicals (12).

Characterization of ethanolic extract: The Central Laboratory for Chemical Analysis at the University of Technology in Iraq conducted analyses using the Fourier transmission infrared (FTIR) (13) and UV/Vis spectrophotometry (UV-vis) (Shimadzu) technologies (14).

Bacteria pathogenic identification and diagnosis: Isolates of *(E. coli*, and *Staph. aurous*) were supplied from laboratory of microbiology. All microbial isolates were identified at species level by the VITEK-2 compact system with (GP) card for gram positive bacteria identification, and (GN) card for gram negative bacteria identification.

Antimicrobial assay for ethanolic extract: The antibacterial activity was assessed by agar well diffusion method (13, 14). According to the manufacturer's instruction, Mueller Hinton agar was prepared and used, 0.1 mL of overnight strain culture (adjusted to 0.5 McFarland turbidity) streaked entirely on the Mueller

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Hinton agar using sterile swab stick. Four wells were bored in the culture medium using a sterile 6 mm diameter cork borer. 0.1 ml of each concentration (0.2, 0.4, 0.6 and 0.8) μgmL^{-1} of ethanolic extract was added to different wells using a sterile micropipette. The negative control had D.W. The observed inhibition zones were measured and recorded in millimeters. This was done in triplicates (15).

Antioxidant Activity by DPPH: By utilizing the DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical, scavenging activities were evolved. Based on the methodology described in the references (16), the ethanolic extract of o*nion allium* plant DPPH radical antioxidant activity was assessed. 500 µL of the DPPH in absolute alcohol was prepared, and 10 µL of ethanolic extract solution (12.5, 25, 50 and 100 μgmL^{-1}) was added to the mixture. The samples were incubated for 30 minutes at 37°C, and the absorbance was measured at 517 nm. A DPPH solution only (500 µL) was prepared as a blank solution. The measurement was carried out three times. According to Equation 1, the antioxidant activity was determined (17, 18).

1) Scavenging activity %= $\frac{OD^{control} - OD^{sample}}{OD^{control}} \times 100$

OD= Optical density

Cytotoxicity Assays: The MTT assay was used to determine the cytotoxic effects. Various concentrations of the ethanolic extract of *O allium* (6.125, 12.5, 25, 50, and 100 μgmL^{-1}) were used. 1×10^4 cells breast cancer AMJ-13 cell line was cultured in RPMI-1640. Once the monolayer was formed after overnight incubation, the extract was applied to the cells. After removing the medium and giving the cells 1.5 hours at 37°C, the viability of the cells was assessed after 72 hours by adding 28 µL of a 2 μgmL^{-1} MTT solution. The remaining crystals were then solubilized in the wells by adding 130 µL of DMSO and incubated at 37°C for 15 min with shaking after the MTT solution was removed (19, 20). A micro plate reader operating at 492 nm measured the absorbency. Using the following Equation 2, the amount of cytotoxicity was calculated.

2) Cytotoxicity activity % = $\frac{OD^{A} - OD^{B}}{OD^{A}} \times 100$

A= Control, B=Sample, OD= Optical density

The obtained data were statically analyzed using an unpaired t-test with Graph Pad Pris.The values were presented as the Mean±SD of triplicate measurements (14).

Results

Screening of extract for chemical compounds: Screening of ethanolic extract *O. allium* plant chemical compounds revealed that the active compound found in the alcoholic extract of *O. allium* include saponins; phenolic compounds; glucosides; flavonoids, and polysaccharides and the assessments are positive as shown in Table 1. As shown in Figure 1, the optical absorption declines severely as wavelength rises, then slightly rises at 400 nm.

The FT-IR spectrum of ethanolic extract *onion allium* plant is shown in Fig 2. The spectrum recorded in the range of 200-3800 cm^{-1} showed the bands 700-900 cm^{-1} due ester(S-OR), 880-995cm^{-1} due C-H&=C-H, 1050-1200 cm^{-1} due thiocarbonyl (C=S),1030-1060 cm^{-1} due sulfoxide (S=O), 1615-1785 cm^{-1} due tocarboxylic acids, and 3660-3600 cm^{-1} due (O-H).

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Table 1. Initial screening of *onion allium* extract

Chemical compounds	Presence (+ve) and Absent (-ve)
Saponins	+ve
Polysaccharides	+ve
Glucosides	+ve
Reducing sugar	-ve
Steroids	-ve
Flavonoids	+ve
Phenolic compounds	+ve

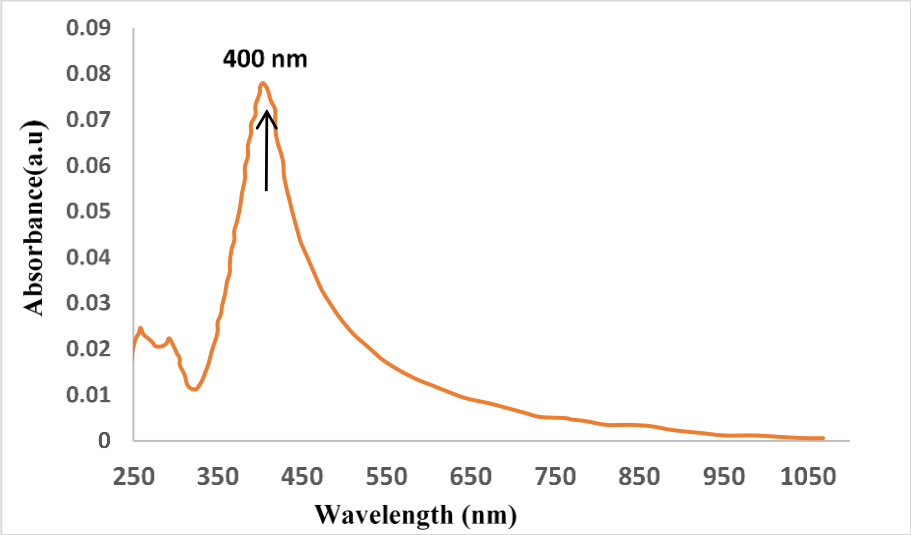

Figure 1. UV Spectrum analyses of ethanolic extract *onion allium* plant

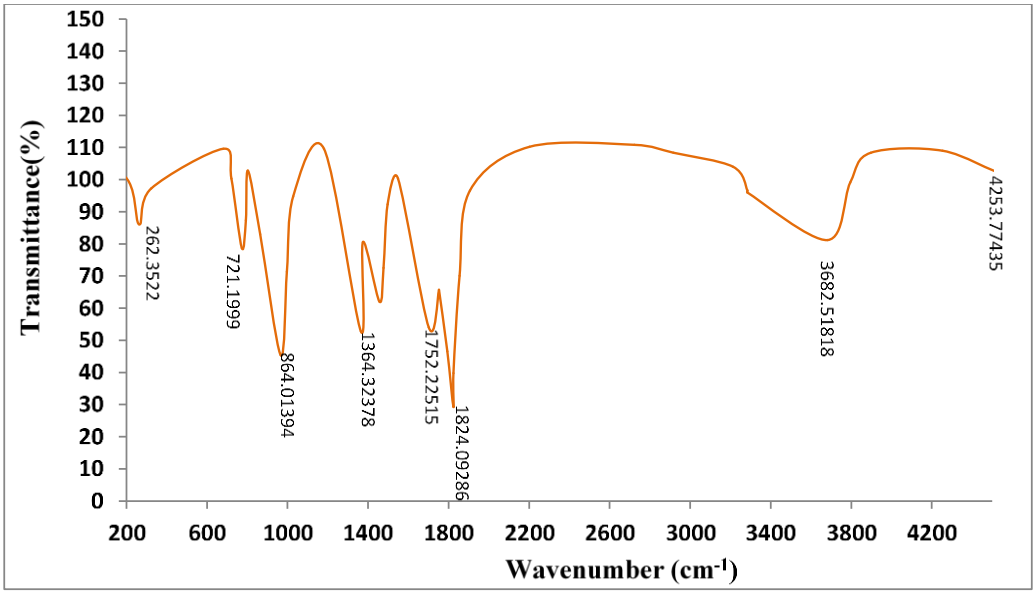

Figure 2. FT-IR spectrum of ethanolic extract *O.Allium* plant

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Antibacterial activity: The evolution-inhibitory effects of ethanolic extract of *O. allium* plant against *S. aureus* and *E. coli* were indicated by the antibacterial properties of the plant at varied concentrations (0.2, 0.4, 0.6 and 0.8 µgmL^{-1}). According to Figure 3, Gram-negative bacteria are more susceptible to ethanolic extract than Gram-positive bacteria. The observed antibacterial effect of ethanolic extract against *E. coli* is shown in Table 2, with a noticeable inhibition zone (12.33±0.38, 13.56±0.45, 16.26±0.42, and 20.50±0.12 mm) for various concentrations (0.2, 0.4, 0.6 and 0.8 µgmL^{-1}). However, regarding the antibacterial outcome of the ethanolic extract against *S. aureus*, the inhibition zones (11.50±0.38, 13.5±0.45, 15.4±0.42, and 17.17±0.33 mm) were recorded for various concentrations (0.2, 0.4, 0.6, and 0.8 µgmL^{-1}), as shown in Figure 3. Table 2 shows that inhibition zone for *E. coli* Gram-negative bacteria is higher compared to *S. auras* Gram-positive bacteria for the same treatments (ethanolic extract of *O. allium* plant).

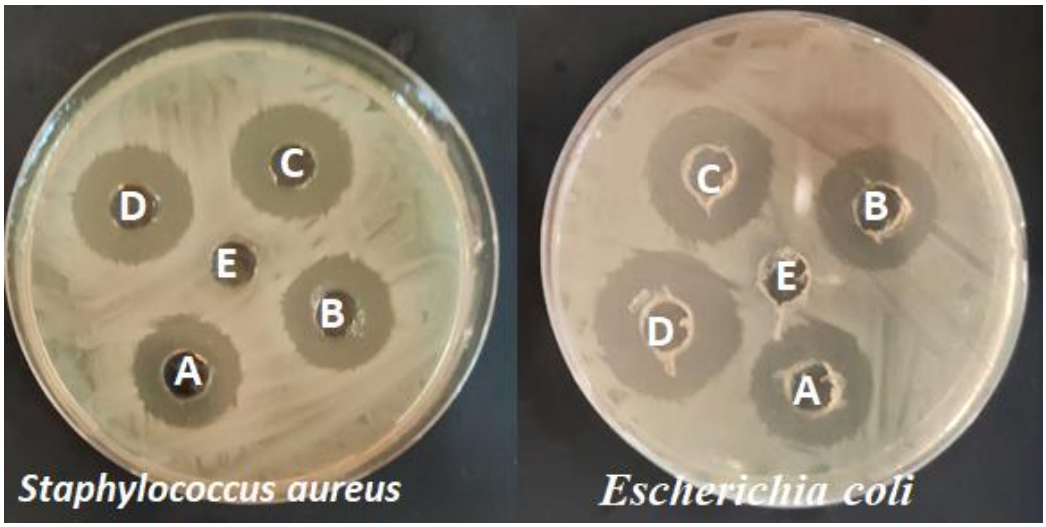

Figure 3. Inhibition zone of ethanolic extract against two pathogens for various concentrations A= 0.2 µgmL^{-1}, B= 0.4 µgmL^{-1}, C=0.6 µgmL^{-1}, D= 0.8 µgmL^{-1}, E= control

Table 2. Antibacterial activity of ethanolic extract for various concentrations

Microorganisms	Concentrations (µgmL^{-1})				
	A	B	C	D	E
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Staphylococcus aureus	11.50±0.38	13.5±0.45	15.26±0.42	16.50±0.25	5.53±0.001
E.coli	12.33±0.38	13.56±0.45	16.26±0.42	20.50±0.12	5.53±0.001

A= 0.2 µgmL^{-1}, B= 0.4 µgmL^{-1}, C=0.6 µgmL^{-1}, D= 0.8 µgmL^{-1}, E=control.

Antioxidant activity: The antioxidant activity of ethanolic extract acts as a scavenger of the DPPH free radical due to a decrease in these radicals, which illustrates the free radical scavenging characteristics exhibited by ethanolic extract of *O. allium*; the extract has higher antioxidant activity, indicating its ability to interact and neutralize free radicals in order to prevent them from causing damage. The assays conducted for ethanolic extract was scavenged proportionally to the concentrations, i.e., at concentrations of 12.5, 25, 50 and 100 µg mL^{-1}. The DPPH free radicals' scavenging capacities were 54.07±0.540%, 65.73±0.041%, 75.59±0.040%, and 91.11±0.015%, respectively (Figure 4). The concentration was read; the findings demonstrated that ethanolic extract significantly decreased the level of DPPH in a concentration-dependent manner, with 100 µgmL-1 being noticeably superior to the other concentrations.

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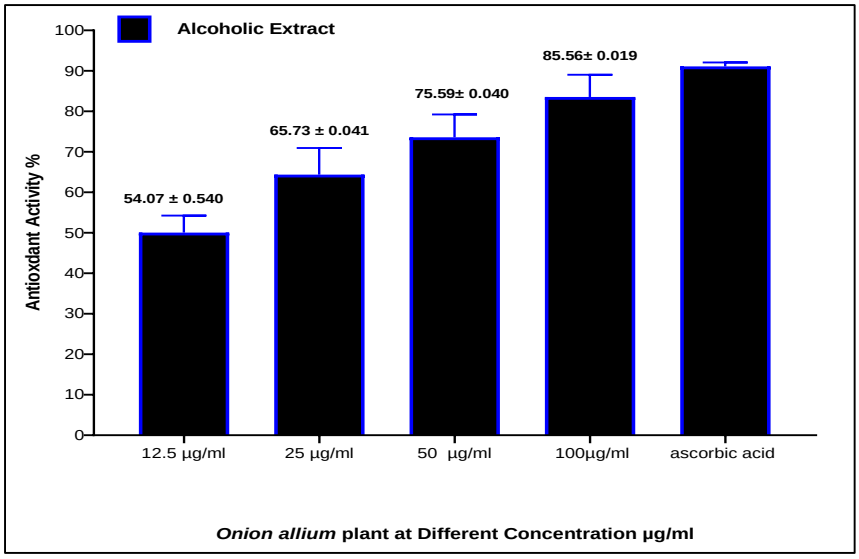

Figure 4. Antioxidant activity of ethanolic extract onion allium plant by DPPH assay

Anticancer activity: The most effective cytotoxic effect (82.66%) was observed at a dose of 100 µgmL^{-1}, while cells were affected at 6.25 µg mL^{-1} 3.33% compared to the control group. This type of activity, on the other hand, was not observed in the normal human breast epithelial cell line; when compared to cancer cells, their normal cell proliferation rate demonstrated a less cytotoxic effect ethanolic extract of *O. allium* plant, indicating that ethanolic extract of *O. allium* plant has improved anticancer efficacy (Figures 5 and 6). The assays conducted for ethanolic extract showed anticancer effect proportional to the concentrations, i.e., at concentrations of 6.25, 12.5, 25, 50 and 100 µg mL^{-1}. The cytotoxic effect capacities were 3.33±0.50%, 12.50±0.41%, 34±0.40%, 47.66±0.50% and 83.66±0.50, respectively (Figure 4). The concentration was read; the findings demonstrated that ethanolic extract significantly decreased the level of cytotoxic effect in a concentration-dependent manner, with 100 µgmL^{-1} being noticeably superior to the other concentrations.

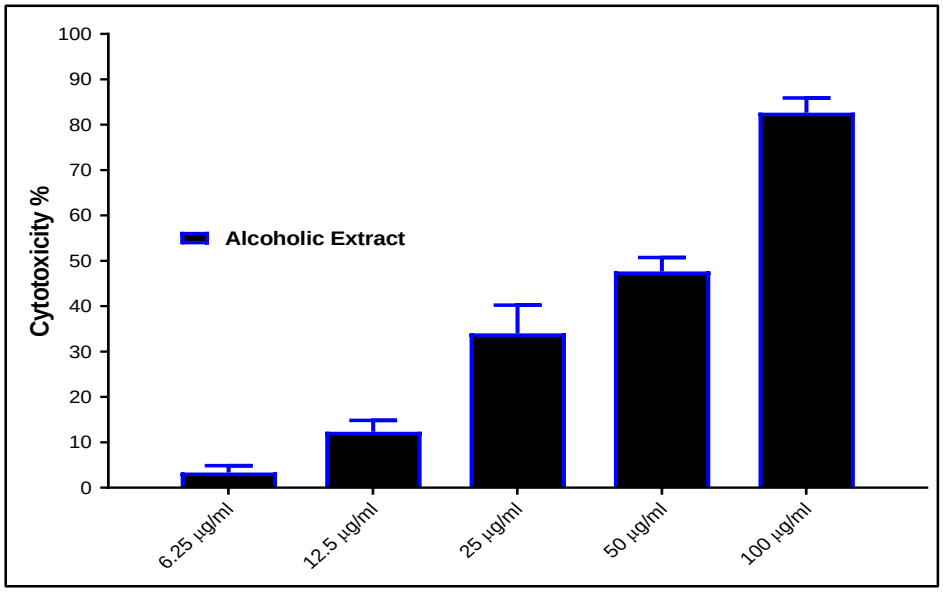

Figure 5. Anticancer activity of ethanolic extract in AMJ-13 cell line

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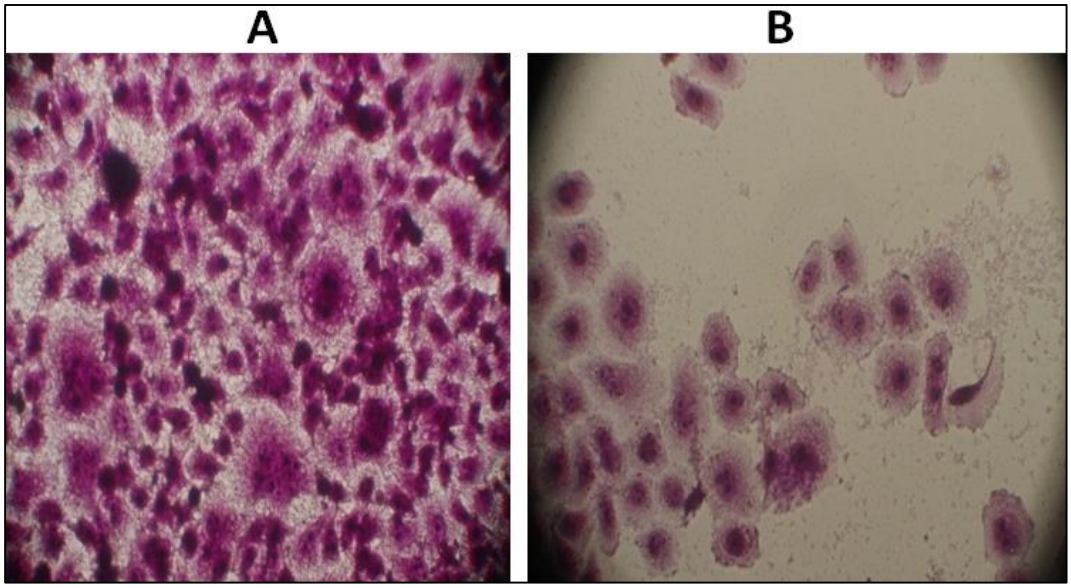

Figure 6. The change morphology in AMJ-13 cell line by ethanolic extract
A= Control cell, B= Treatment cell

Discussion

Differences between the cell wall structures of Gram-negative bacteria and Gram-positive bacteria explain why the prepared onion allium plant responds differently to antibacterial treatment. Several mechanisms in ethanolic extract of *O. allium* plants on bacterial cells must be investigated, despite the fact that its antibacterial activity has not yet been identified. However, the researchers discovered that employing *O. allium* enhances the generation of Reactive Oxygen Species (ROS) in bacterial cells (7). Based on the features of the bacteria cells, the antibacterial activity of the onion allium plant appears to differ. The cellular walls of *O. allium* plants appear to have an impact on their antibacterial action (8). According to research by Hasan et al (10), the antibacterial capabilities of the *O. allium* plant are similarly impacted by Lanzotti et al (21).

The DPPH measurement has been widely used with a variety of extract concentrations to assess the efficacy of the extract as an antioxidant. Additionally, it provides quick and repeatable outcomes. Six recently published methodologies are compared for estimating antioxidant capacity and have shown which DPPH procedures are more expedient, simple, and accurate (18).

This suggests that allium extract from onions can be taken as dietary supplements to stave off illnesses. Antioxidants are also used extensively in the industry (17). Determination of total antioxidant power, there are numerous documented methods that can be split into two categories: electron transfer testing (ET) and hydrogen atom transfer testing (HAT). The HAT-based tests, including the ORAC test, employ a dynamic reaction system in which the substrate and scavenger vie for radicals produced thermally. There is an identified and established method that is sufficient and thorough for determining a substance's effectiveness and researching its antioxidant properties. When measuring the antioxidant strength using multiple types of measurement techniques, several mechanisms and actions of various antioxidants can be considered (16). In the present research, DPPH procedures were used to assess a plant extract's capacity to eliminate free radicals.

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This decrease in cell viability as onion allium plant concentration increased indicates that more onion allium plant were able to accumulate within cells, stressing and eventually killing them. Bio kinetics and toxicity are heavily influenced by size, morphology and surface functioning, as demonstrated by the dose-dependent induction of apoptosis. According to the current findings, cell death caused by onion allium plant could be due to apoptosis or necrosis. The mechanisms by which onion allium plant cause cell death must be investigated. Apoptosis induction by anticancer drugs via their antitumor effects on cancer cells is a significant phenomenon in cancer chemotherapy (18, 19, 22-26). The current study's findings are consistent with those of by Zamri et al. and Al-Majedy et al. (20, 27), who noticed that as extract concentration increases, cell viability decreases.

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