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CYTOTOXICITY, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACT OF *QUERCUS INFECTORIA* PLANT

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Abstract

Antimicrobial, cytotoxic and antioxidant effects of alcoholic extract for the *Quercus infectoria* plant were studied. The antimicrobial activity was investigated against some pathogenic microbes (*E.coli*, *Staphylococcus aureus*, and yaest *Candida albicans*). Results showed the highest effect for crud extract in the growth of *Candida albicans* by zone of inhibition diameter reached to (37.33±0.58), followed by *Staphylococcus aureus* by diameter of inhibitionb zone reached to (31.33±2.30 mm), finally (24.33±0.58) in *E. coli*. The results also demonstrated that treatment with alcoholic extract significantly inhibited the growth of cells (MCF-7 cell line) in a concentration dépendent manner. The results indicate the plant *Quercus infectoria* can be considered as a particularly valuable source of effective antiproliferative and cytotoxic agents, Also the extract have a higher antioxidant activity by DPPH assay.

Keyword **:** Biological activities; Medicinal plant; Alcoholic extract

Introduction

Q. infectoria, (Figure 1), usually recognized as oak, is a small shrub found in Asia Minor and Iran. It is a small tree or hrub growing to 4 to 6 feet tall, crooked, with smooth and bright leaves, acorn long and narrow, scaly and downy, it's used since ages as a home therapy for sore throat and chronic diarrhea in both rural and urban areas from India (Dhiman, 2006). Various plans. Species are initiated in Iran, Iraq and Turkey (Anonymous, 2005). Galls contain 50-70% of the tannin (Ewans, 2002). Galls extracts have significant anticancer effect (Hasmah *et al.* (2010), gall Considered as one of the active phytotherapeutic agents in against bacterial infections. Many studies have been developed on the active compounds in this plant, because of its high effectiveness against microbes (Wan Nor Amilah, 2014), and this has been used it as an antiseptic (Mahmoud A. El-Sedfy *et al.*, 2018). This study aimed is to determine the some biological activities of *Q. infectoria* Plant.

Fig. 1 : Fruits of *Q. infectoria*

Materials and Methods

Extract Preparation

The extraction was done by soxhelet, and solvent 70% ethanol, then the solvent was totally removed by using a rotary evaporator to get a semi-solid form, then transferred to oven for obtain the crud extract, and stored at 4^0 C until used.

Phytochemical Screening of *Q. infectoria* extract:

The extract was exposed to initial phytochemical screening for the determination of several phytoconstituents according to (Ghafour *et al*., 2010).

FTIR assay:

Fourier transmission infra-red (FTIR), and UV Spectrum (Shimadzu) analyses were performed in the Lab of Iben Sena center/ University of Baghdad, Iraq.

Antioxidant activity: DPPH assay:

Antioxidant activity of *Q.infectoria* extract was measured using stable DPPH radicals with minor adjustments according to (Tailor and Goyal, 2014). Extract were used to investigate the scavenging activity. Every sample was mixed with DPPH solution and then completes the quantity to one particular mL using ethanol. The decline in absorbance was measured at 517 nm. Scavenging activity computed according to the equation formula:

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Preparation of bacterial isolates:

Microbial isolates were taken from isolated specimens. The microbes were seeds overnight at 37 ^{0}C on NB to preparation the suspensions cell. The microbial suspensions cell was homogenized and attuned to 0.5 McFarland standards (5 × 105 CFU/mL) by spectrophotometry.

Antimicrobial assays:

Isolates were obtained from the Biotechnology Branch/Applied Science Department at the University of Technology. Agar diffusion method was used to detect antimicrobial activity of plant. After the microbes were diffused with a spreader on MH agar, plant extracts were melted in 10% DMSO, and wells that were 6mm in diameter were filled with concentrations of (20,60,80,and 100) mg mL^{-1}, with 10% DMSO as a negative control. After incubation in 37ºC for 24h, the ZOI was measured around each well and compared with the control and conducted in in triplicate (Nehia *et al*., 2019).

Radical scavenging activity of *Q. infectoria* plant:

Detection of the toxicity: Maintenance of cell cultures:

Lung cancer cell line (Al-Shammari *et al.,* 2015). were took from the Iraq biotech CBU and preserved in RPMI-1640 enhanced by 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C.

Cytotoxic Activity:

This assay was done according to (Al-Shammari *et al*, 2016).

Statistical Analysis

All of the tests were conducted in triplicate. Data were reported as means ± standard deviation.

Results and Discussion

Preliminary Phytochemical Screening of plant extract:

Preliminary Phytochemical Screening of *Q.infectoria* extract Based on the preliminary screening, Terpenoids, Steroids, and Glucosides were absent. Color change such as dark green color indicates presence of tannins and phenolics, reddish brown interface (terpenoids and glycosides), greenish (steroids), yellow to colorless (flavonoids) respectively. Formation of precipitate such as red precipitate indicates presences of phlobatannins or reducing sugar, yellow or reddish brown (alkaloids) and yellow precipitate (flavonoids) while persistence of frothing indicates presences of saponins (Table1). Results in (Figure 2) showed that the high severe, band (-3223cm^{-1}), which signify refer to (OH) groups, band of 1705.07cm^{-1} that refer to C=O group .Also bands of (1614cm^{-1}, 1537cm^{-1}) refer to amine and nitrate group , bands of (1450.47cm^{-1}) that refer to presence of C-H alkenes group. Band of 1207.44cm^{-1} refer to C-O group. bands of (1087 cm^{-1}) that refer to chloride groups, 2727.35 cm^{-1} that refer to O-H stretch group ,the band 1539.20 cm^{-1} that refer to N-H amide (Whittaker , 2000).

Table 1 : Phytochemical Screening of *Q.infectoria plants*

Sample *Q. infectoria*	Constituent
+	Tannins
+	Saponins
+	Phenolic compounds
+	Phenol
-	Terpenoids
-	Steroids
-	Glucosides
+	Alkaloids Wagner's test
+	Flavonoids Ferric chloride test
+	Volatile oil
+	Comarines
+	Resins

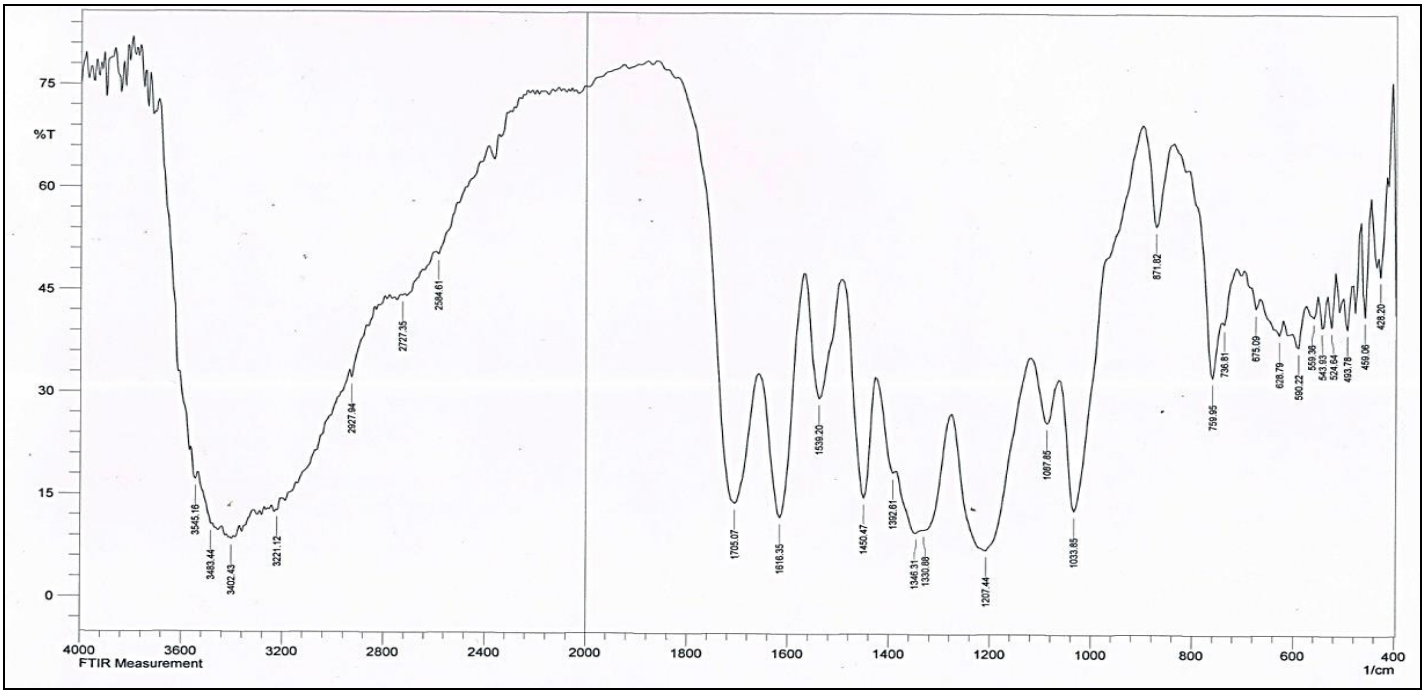


Fig. 2 : FTIR test to *Q. infectoria* gall extract

Evaluation of Antimicrobial activity:

Q. infectoria extracts effects was studied against three types of pathogenic microbes. The effects of ethanolic extract were varied on the growth of pathogenic microbes. Highest effect observed on the growth of *C. albicans* by zone of inhibition reached to (37.3333 ± 0.58 mm), followed by *S.aureus* (31.33±2.30mm), finally in *E. coli* with a diameter of zone of inhibition that reached to (24.33 ± 0.58), (Figure 3 and Table 2).

Table 2 : The effect of crud extract against some pathogenic microbes each value represents the mean ± SD of 3 trials.

Microorganisms	Concentrations µg / ml^{-1}			
	100	80	60	40
E. coli	24.33±0.58	27.33±0.58	19.33±0.58	17.33±0.58
S.aureus	31.33±2.30	34.33±1.15	26.66±0.58	24.00±1.73
C. albicans	37.33±0.58	35.33±0.58	32.33±0.58	28.33±0.58

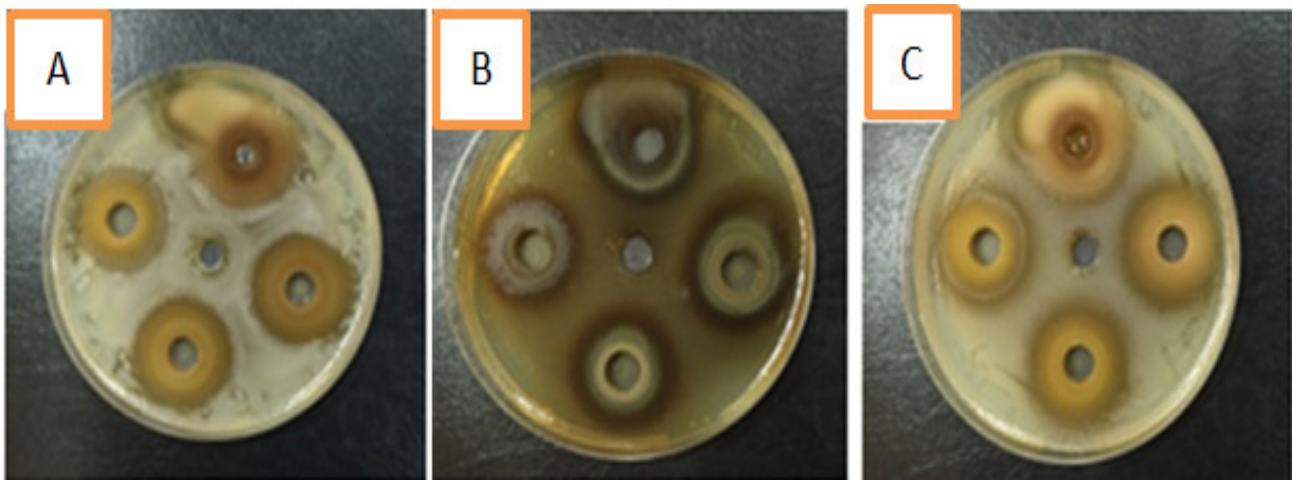


Fig. 3 : Antimicrobial activity of *Q.infectoria* alcoholic extract toward. A- *E.coli,* B- *S.aureus* , and C - *C.albicans*

Tannins attend as natural protection device towards infections, also can be used in some inflammatory settings (Adel and Muhammed, 2010). The antimicrobial activity is mostly due to the occurrence of tannin which is the main component present in this plant, It is a hydrophilic composite frequently produce by hydrophilic organic solvent and prevents the growth of microbes by producing molecules with microbial enzymes complex, changing the bacterial metabolism by inhibition the oxidative phosphorylation and decreasing iron conc. through precipitation with various nitrogen containing the protein groups (Scalbert, 1991).

Antioxidant activity

DPPH assay estimates the hydrogen-donating capability of the chain-breaking antioxidants, that are able to offer H^+ to free radicals, leading to nontoxic kind and inhibition of the propagation point of lipid oxidation. The antioxidant activity of gall extract as a scavenger of the $DPPH^+$ radical due to reduction in these radicals (Figure 4) which shows free radical scavenging characteristics exhibited by *Q.infectoria* plant**.** Extract thus indicates its ability to interact and neutralize free radicals, thus preventing them from causing damage. This implies that *Q. infectoria* plant can be used as dietary supplements for the prevention of diseases such as cancer. Antioxidants also have many industrial uses, such as preservers in food and cosmetics. (Dabelstein *et al.*, 2007).

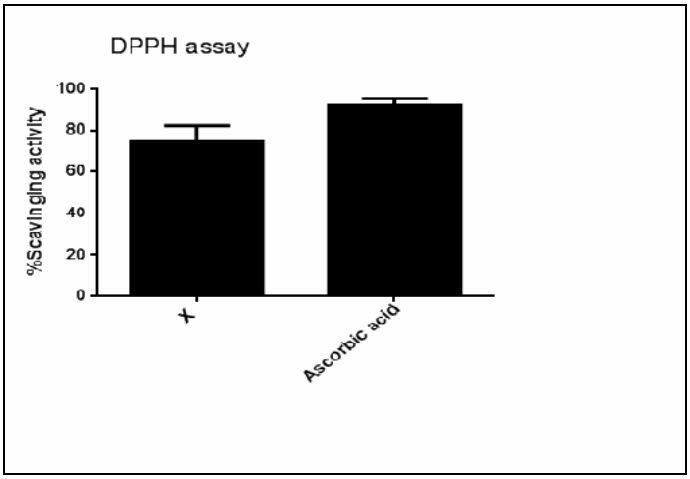
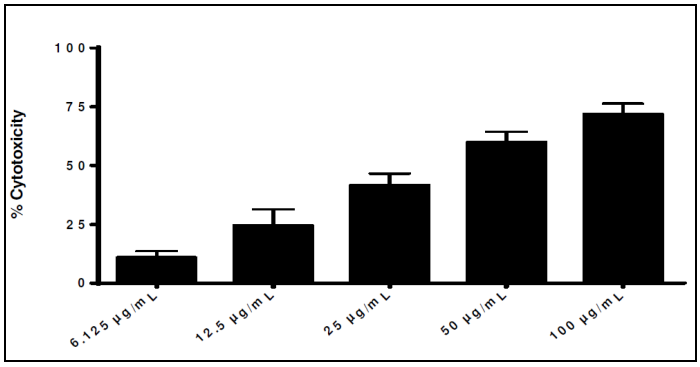


Fig. 4 : The antioxidant activity of Q. *infectoria* plant

Anticancer activity:

The cytotoxic activity was examined as shown in (Figure 5). Our results showed the *Q. infectoria* substance to prevent proliferation of tumer cells and its conc. dependent manner. Results illustrated treatment of the cells with *Q. infectoria* significantly reduce the growth of cells in a concentration dependent manner. The results indicate that *Q. infectoria* is considered to be a source of effective anti-proliferative, also cytotoxic agents. Crud extract of *Q. infectoria* is able to killing tumor cells in the human body by inhibiting and inducing apoptosis effects. In this study, the anti-tumor effect of lung cancer cells was investigated using the crude alcoholic extract of clove plant. Drugs of anticancer exert their antitumor effects against cancer cells by inducing apoptosis that is an significant phenomenon in cancer chemotherapy (Ghassan *et al.*, 2013). Compared to the control nuclei, crud extract treated cells showed condensed and fragmented nuclei. It was observed that level of apoptotic cell death was maximum. Our results supports the medical usage of these plant which owns both important antimicrobial, also the effects on the cancer cell line suggested that it could be used in avoidance of cancer (Aggarwal and Shishodia, 2006). Tumor is one of the most important reasons of death in the world. Resistance to the drugs used as anticancer therapies has been recently shown which highlights the urgent need to conduct further research to discover plant-derived substances that act as anticancer. Many plants, vegetables, herbs and spices might have the potential use in medicine as a source to the prevention of cancer, which necessitates further studies of these plants to know their biological properties and therapeutic potential (Salomons *et al*., 1999).



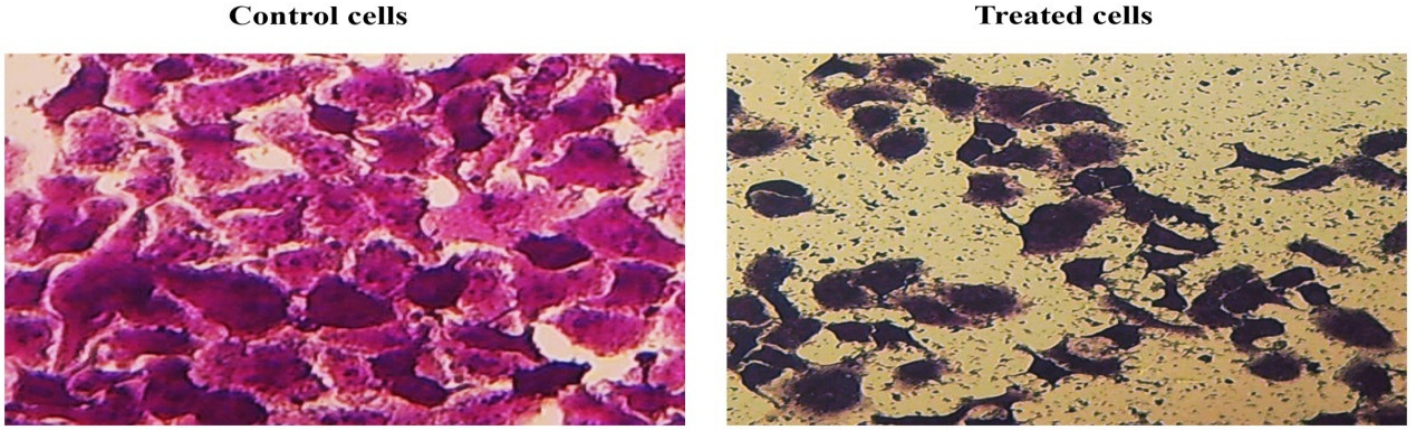


Fig. 5 : Cytotoxic effect of *Q.infectoria in* MCF-7 cell line

Conclusion

Q. infectoria galls have been exposed to quite wide phytochemical, experimental and clinical investigations. The scientific studies have showed most of the claims of traditional medicines.

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