

**ANTICANCER, CYTOTOXIC EFFECT OF *TAMARIX APHYLLA*, AND ANTIBACTERIAL SCREENING EFFICIENCY AGAINST MULTIDRUG-RESISTANT HUMAN PATHOGENS**

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**ABSTRACT**

**Objectives:** The present study aimed to investigate the anticancer, cytotoxic effect of *Tamarix aphylla*, and antibacterial effectiveness against 10 pathogenic bacteria that cause common and sometimes serious infections in human and animals.

**Methods:** *T. aphylla* fresh and disease-free leaves were collected from the different geographical regions of Saudi Arabia. The anticancer, cytotoxic effect of *T. aphylla* leaves, and antibacterial screening efficiency against multidrug-resistant human pathogens were investigated *in vitro* using Vero cells as a normal cells and MCF-7 (breast adenocarcinoma cells).

**Results:** *T. aphylla* leaf extracts exhibited a low cytotoxic effect on Vero cell line at high concentration, with an 50% cytotoxicity concentration value of >1000 µg/ml. The methanolic extract inhibited MCF-7 cancer cells in a concentration-dependent manner. The methanol and ethanol extracts showed antibacterial activity with variable inhibition effects and differences in their activities against tested pathogenic bacteria ranging from very high inhibition (20.7±1.3 mm) to low (4±0.6 mm). The minimum inhibitory concentration of methanol and ethanol results showed no significant differences.

**Conclusions:** The findings of this study conclude that the *T. aphylla* leaf extract had lower toxicity on normal cell line (low toxic plant) and significantly inhibited the growth of cancer cells. *T. aphylla* has potential antibacterial biomolecules against multidrug-resistant human pathogens.

**Keywords:** *Tamarix aphylla*, Leaves, Cytotoxic activity, African green monkey kidney cells (Vero) and breast adenocarcinoma cells (MCF-7), Anticancer effect, antibacterial.

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**INTRODUCTION**

The use of medicinal plants in the community increased in the recent years for curing some health-related diseases [1,2] and has been generally used in many different parts of the world [3].

The medicinal plants possess antimicrobial effects which have received more attention in the past few years [4]. In some communities, consuming herbal drugs and traditional medicine is somewhat more popular because of cultural acceptability and belief that being natural, and also, it is considered as the cheapest and safer sources for discovering new effective antimicrobials [5]. Medicinal plant has been identified by the World Health Organization (WHO) as any plant which contains active compounds that can be used for therapeutic tools or which are pioneers for the synthesis of effective safe drugs [6]. Several drugs and pharmaceuticals constitute approximately 40% plant source [1]. Infectious diseases are a common major global challenge for medical, scientific communities, health organizations, and pharmaceutical companies around the world. It has been estimated that 50,000 deaths every day especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the many available antibiotics [5,6]. Cancer is a global major health problem, it is the second leading cause of death Worldwide [7,8], and about 14 million new cases and 8.2 million cancer-related deaths were reported in 2012 [7-9]. The global incidence of cancer is more 11 million with up to 7.6 million deaths, in which 72% of these deaths were in low-income and middle-income countries [9,10], and is expected to increase to an incidence of 15.5 million with 11.5 million deaths by 2030 [7-9].

The WHO reported that 80% of the population worldwide practices herbal medicine for cure primary health cases [11]. Currently, 50% of all

drugs in the market and 74% of the most important drugs are derived from phytochemicals [9,11]. Currently, more than 60% of commercially available anticancer drugs are derived from natural sources, including plants, marine organisms, and microorganisms [9,12]. To date, more than 3000 different plant species have been used in the treatment of different types of cancer [9,11]. Extensive research on medicinal plants has identified several botanical natural compounds that have chemopreventive potential [12]. Plant bioactive molecules and phytochemicals could prevent more 20% of cases of cancer and may potentially prevent approximately 200,000 cancer-related deaths annually. Due to their safety, low toxicity, and antioxidant properties, several plants are being investigated for the prevention of cancer [9,10]. Tamaricaceae is comparatively a small family containing 4 genera and about 120 species of flowering plants [15]. Members of the family are mostly temperate and subtropical, growing in marine or sandy habitats. Geographically, this plant distributed in the Mediterranean, North Africa, and south-western Africa through the Arabian Peninsula to Central and South Asia [15].

*Tamarix* is distributed in Saudi Arabia with eight species, namely *Tamarix mascatensis* Bunge., *Tamarix ramosissima* Ledeb., *Tamarix nilotica* (Ehrenb.) Bunge., *Tamarix aphylla* L., *Tamarix tetragyna* Ehrenb., *Tamarix aucheriana* Decne., *Tamarix pyconocarpa* DC, and *Tamarix passerinoides* Del [13,14]. Several studies revealed that some *Tamarix* species have been used as a diaphoretic, diuretic, and hepatotonic and to treat liver disorders, to relieve headache, anti-inflammatory, ease prolonged, or difficult labor, and to treat sores and wounds [13,15,16]. Different parts of *Tamarix* are used; the leaves and young branches for edema of the spleen are mixed with ginger for uterus infections, while bark part is used as lotion against lice, eyes sore, and hemorrhoid [15]. *Tamarix* species contain different classes of natural products [13,16]. Various secondary metabolite compounds have been isolated as a

carbohydrate, phenols, flavonoids, terpenoids, steroids, tannins, and cardiac glycosides, and due to the existence of these compounds, the *Tamarix* has been shown to have antibacterial, antifungal, antiseptic, and anti-parasitic actions [2,13,15]. It is, therefore, very necessary that the search for newer medicinal based antibiotic sources to be a continued process.

The present study aims to investigate the anticancer, cytotoxic effect of *T. aphylla*, and antibacterial screening efficiency against 10 pathogenic bacteria that cause common and sometimes serious infections in human and animals.

## METHODS

### Collection of *T. aphylla* samples

*T. aphylla* fresh and disease-free leaves were collected from the different geographical regions of Saudi Arabia. The taxonomic and morphological identification of the plant material was confirmed by Herbarium Center at the Botany and Microbiology Department, King Saud University. The plant was morphologically identified by Herbarium Center at the Botany and Microbiology Department, King Saud University. The plant samples were sealed in sterilized polythene bags, were brought to the laboratory, and were washed thoroughly 2–3 times with running tap water and once with sterile water, left at room temperature for 3–5 days in the dark, then oven-dried for 1 h at 160°C, and placed at 37°C till complete drying, and then, whole plant was ground using sterile coffee grinder into a fine powder.

### Extraction with ethanol and methanol

The prepared powder was soaked in each of ethanol and methanol solvents (10 g in 100 ml) and extracted for 24 h at room temperature with shaking at 150 rpm. After filtration through a Whatman no. 4 filter paper, solvents were evaporated under reduced pressure using R-215 Rotary Evaporator (Sigma-Aldrich) until dryness. The solvent-free brownish crude extract thus was obtained and was re-suspended in dimethyl sulfoxide (DMSO) or phosphate-buffered saline (PBS) to a final stock concentration of 50 mg/ml or 100 mg/ml. All extracts were stored at 4°C in airtight dark bottles till use [17,18].

### Test pathogenic bacterial species

Bacterial species used in this study (Table 1) were clinical isolates obtained from the Botany and Microbiology Department, Faculty of Science, King Saud University. All of the bacterial species were grown and maintained on Mueller-Hinton agar or trypticase soy agar media at 37°C pH (7.3±0.2). All bacterial species were identified and characterized by culturing in the specific appropriate media followed by the rapid testing (Gram's stain, catalase, oxidase, coagulase, and bile solubility) and the biochemical testing (indole, methyl red, Voges-Proskauer, citrate, triple sugar iron, oxidation/fermentation, urease, and nitrate reduction).

### Bacterial species inoculums

The tested bacterial species were first inoculated into tubes which contain Mueller-Hinton Broth separately and incubated at 37°C for 18 h. Each of the cultures was then adjusted to 0.5 McFarland Turbidity

Standard at 1–2×10<sup>6</sup> CFU/mL and inoculated (0.1 ml each) onto Mueller-Hinton agar plates (diameter: 15 cm).

### Maintenance of cell lines

African green monkey kidney cells (Vero) and breast adenocarcinoma cells (MCF-7) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY), 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml) (Sigma, St. Louis, MO). Cells treated with medicinal plant extracts were kept in maintenance medium containing 1% FBS, L-glutamine, and antibiotics. Cells were incubated at 37°C with 5% CO<sub>2</sub> for 3 days.

### Evaluation of Vero cell morphology

Monolayer cultures of monkey kidney cells (Vero) (80–90% confluence) were prepared in 96-well plates. After removal of culture medium, cells were washed twice with PBS. Two-fold serial dilutions of the methanol, ethanol, and water extracts were prepared in maintenance medium starting from the concentration of 2500 to 4 µg/ml and added to cells in triplicates. Wells that received ethanol 70% or DMSO were served as positive controls and those that received maintenance media only were served as negative controls. All cultures were kept at 37°C in CO<sub>2</sub> incubator for 72 h with daily observation for morphological changes under phase-contrast inverted microscope equipped with a digital camera (Olympus IX51, Tokyo, Japan) at ×20 and ×40. Cellular alterations were recognized in the form of cell rounding, granulation, vacuolation, degeneration, and lysis, as well as detachment of the monolayer. The minimal toxic concentration (MTC) was identified as the least concentration that induces toxic effect(s) on culture cells as detected microscopically 3–4 days post incubation as described by Abdul *et al.* [21].

### Antiproliferative and cytotoxic effect assay

The cytotoxic effect of the extracts of *T. aphylla* leaf against Vero cells as a normal cells and MCF-7 (breast adenocarcinoma cells) was determined by a rapid colorimetric assay, using cell titer-blue (CTB) reagent (Promega, Madison, WI). The color is quantified using multi-well ELx880 microplate reader (ELISA reader) (BioTek, Winooski, VT) with wavelength 570 nm (ELISA reader). Briefly, 180 µl of cell suspension (2×10<sup>4</sup> cell/ml) was seeded in 96-well microplates except for the first row which contained only 180 µl of DMEM which was considered as the blank. After 24 h of incubation in the previously mentioned conditions, the cells were treated with a 20 µl of two-fold dilution series of plant extracts and incubated at 37°C for 72 h in a CO<sub>2</sub> incubator. After completion of the treatment period, the 20 µl of CTB reagent (Promega, Madison, WI) was added to each well. For the positive control, 20 µl of DMSO was added instead of extracts. After 4 h of incubation at 37°C, the optical density was measured in all plate wells using ELx880 microplate reader (BioTek, Winooski, VT) with wavelength of 570 nm [21]. The cell viability was measured in each well using the following formula [21]:

$$\text{Cell viability} = \frac{\text{OD}(\text{assay well}) - \text{OD}(\text{positive control})}{\text{OD}(\text{cell control}) - \text{OD}(\text{positive control})} \times 100$$

The 50% cytotoxicity concentration (CC<sub>50</sub>) was calculated as the concentration of the plant extract that induced reduction in cell viability to 50%.

### In vitro antibacterial bioassay

Antibacterial activity of the ethanol, methanol, and aqueous extracts was screened by agar well diffusion method as described by Gawade and Farooqui [20] and Abdul *et al.* [21]. Briefly, the bacterial suspension was swabbed uniformly to the Petri dishes containing 20–30 Mueller-Hinton Agar (MHA), and the inoculum was allowed to dry for 5–10 min. Nine wells of 6–8 mm in diameter were made in the surface of inoculated MHA using sterile cork borer for different concentrations of the extract and controls. The 50 µL from each extract (1, 5, 10, 50, 100, 200, 250, and 500 mg/mL) was added into each well on the MHA plate and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated

**Table 1: Bacterial species, media, and cultivation conditions**

No.	Bacterial species	Media and cultivation conditions
1	<i>Klebsiella pneumoniae</i>	TSA+YE or MHA, 37°C
2	<i>Proteus mirabilis</i>	TSA+YE or MHA, 37°C
3	<i>Enterococcus faecalis</i>	TSA+YE or MHA, 37°C
4	<i>Staphylococcus aureus</i>	TSA+YE or MHA, 37°C
5	<i>Salmonella typhi</i>	TSA+YE or MHA, 37°C
6	<i>Streptococcus pyogenes</i>	TSA+YE or MHA, 37°C
7	<i>Shigella sonnei</i>	TSA+YE or MHA, 37°C
8	<i>Pseudomonas aeruginosa</i>	TSA+YE or MHA, 37°C
9	<i>Listeria monocytogenes</i>	TSA+YE or MHA, 37°C
10	<i>Bacillus subtilis</i>	TSA+YE or MHA, 37°C

TSA: Trypticase soy agar, YE: Yeast extract, MHA: Mueller-Hinton agar

at 37°C for 24 h. After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the tested bacterial species) around each of the extracts. Reference antibiotic disc was placed on the agar surface as positive control. Sterilized distilled water was served as negative controls in a separate well. These studies were performed in triplicate.

#### Quantitative antibacterial activity assay by minimum inhibitory concentration (MIC) and minimal bacterial concentration (MBC)

The MIC of the aqueous, ethanol, and methanol extracts was determined for the tested bacterial species in triplicates at varying concentrations by tube dilution method. A tube containing 1 ml of Mueller-Hinton broth was inoculated with a loopful of the tested bacteria previously diluted to 0.5 McFarland turbidity standard. A tube containing Mueller-Hinton broth only was seeded with the tested bacteria to serve as a control. All the tubes were then incubated at 37°C for 24 h and then examined for growth by observing turbidity. The MBC of the plant extract on the tested bacterial species was carried out according to Ajaiyeoba *et al.* [24]. Briefly, 1 ml from the MIC tubes showing no growth was subcultured on to MHA plate and incubated at 37°C for 24 h. The MBC was defined as the lowest concentration of bacteria that showed no bacterial colony. All samples were examined in triplicate.

#### Statistical analysis

Microsoft Excel (2007) data sheets and GraphPad Prism version 6 were used to analyze the data.  $CC_{50}$  values, which is the concentration that kills 50% of the test cells. All data were presented as means  $\pm$  standard deviation.

## RESULTS

### Antibacterial activity

The result of the antibacterial activity of the extracts obtained from *T. aphylla* leaves against tested pathogenic bacteria that cause common and sometimes serious infections in human and animals is shown in Fig. 1. The diameter of all inhibition zones was measured and compared. The methanol and ethanol extracts showed antibacterial activity with variable inhibition effects and differences in their activities against tested pathogenic bacteria ranging from very high inhibition (20.7  $\pm$  1.3 mm) to low (4  $\pm$  0.6 mm). Antibacterial activities were expressed as mean inhibition diameter zones of the three replicates in millimeters (mm). Zone of inhibition does not include the diameter of the well. Both methanolic and ethanolic extracts of *T. aphylla* leaves showed relatively similar antibacterial activity and inhibition effects against Gram-positive and negative bacteria. Highest antibacterial activity was observed with ethanol and methanol extract against

*Klebsiella pneumoniae* (17  $\pm$  0.7 mm and 16  $\pm$  0.6 mm), *Streptococcus pyogenes* (14.7  $\pm$  0.6 mm and 14  $\pm$  0.6), *Shigella sonnei* (13  $\pm$  0.7 mm and 13  $\pm$  0.7), and *Enterococcus faecalis* (12  $\pm$  0.5 mm and 11  $\pm$  0.6 mm), respectively, while the modest antibacterial activity was showed against *Salmonella typhi* (10  $\pm$  0.7 mm and 9  $\pm$  0.7 mm), *Staphylococcus aureus* (10  $\pm$  0.7 mm and 10  $\pm$  0.7 mm), *Pseudomonas aeruginosa* (9  $\pm$  0.7 mm and 5  $\pm$  0.7 mm), *Bacillus subtilis* (8.6  $\pm$  0.6 mm and 8.6  $\pm$  0.6 mm), and *Proteus mirabilis* (7  $\pm$  0.5 mm and 7  $\pm$  0.5 mm), respectively, whereas the lowest activity was observed against *Listeria monocytogenes* with inhibition zone about 3  $\pm$  0.6 and 1.5  $\pm$  0.6 mm, respectively (Fig. 1). The methanol and ethanol extract results showed overall similar antibacterial activity against tested organisms. Noticeably, only a small inhibition was observed with methanol extract against *L. monocytogenes* (Fig. 1).

### The MIC

The MIC was determined for *T. aphylla* extracts which were found active in the antibacterial activity evaluation (Fig. 1). The MIC ranged from 1 to 20 mg/ml (Fig. 2). The MIC of methanol and ethanol results showed no significant differences. The MIC of methanol extract was 20, 20, 15, 10, 10, 10, 5, 5, 5, and 1 mg/ml for *K. pneumoniae*, *S. pyogenes*, *S. sonnei*, *E. faecalis*, *S. typhi*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *P. mirabilis*, and *L. monocytogenes*, respectively, while the MIC values of ethanol extract were 22, 20, 15, 15, 15, 10, 10, 5, 5, and 1.5 mg/ml for *K. pneumoniae*, *S. pyogenes*, *S. sonnei*, *E. faecalis*, *S. typhi*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *P. mirabilis*, and *L. monocytogenes*, respectively (Fig. 2).

### Cytotoxicity of *T. aphylla* extracts

The cytotoxic activity was evaluated *in vitro* according to its effect on cell morphology (microscopic examination) and the metabolic reduction of CTB reagent (colorimetric assay) Vero cells. The multiple concentrations of *T. aphylla* leaf extracts were used and  $CC_{50}$  doses were calculated. *T. aphylla* leaf extracts exhibited low cytotoxic effect on Vero cell line at high concentration, with an  $CC_{50}$  value of >1000  $\mu$ g/ml. The MTC colorimetric assay was determined for which the value was 2000  $\mu$ g/ml. It was also shown that all treatments displayed a percentage of growth inhibition activity in a dose-dependent manner. The results of the cytotoxicity evaluation against Vero cell of *T. aphylla* leaf extracts revealed that this plant might be non-toxic plant.

### Phase-contrast microscopy for morphological analysis

Morphological change investigation using phase-contrast microscopy revealed that *T. aphylla* leaf extracts decreased the number of cells and induced cell shrinkage and cell detachment of the monolayer surface of cells treated with high concentration 2500  $\mu$ g/ml (Table 2). Morphological alterations and cytopathic effect forming of Vero cells

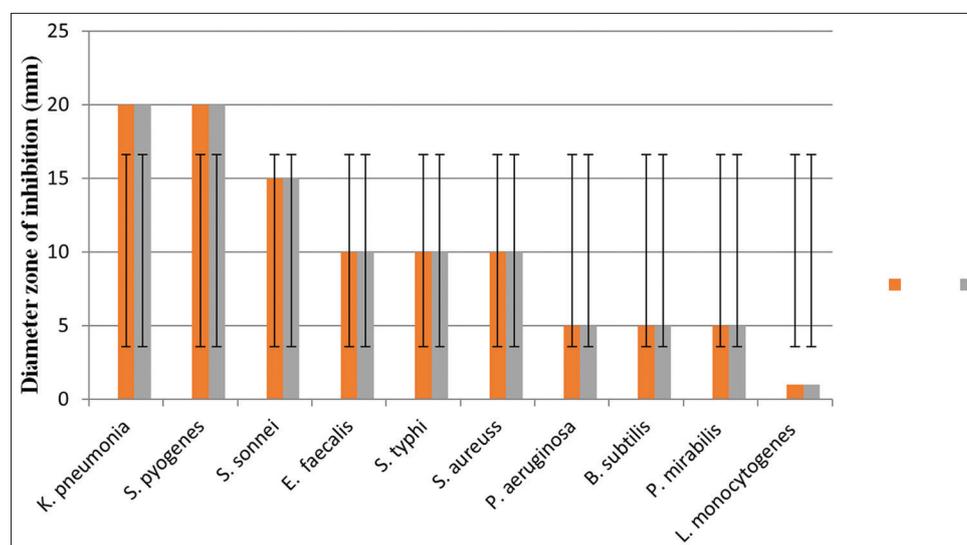


Fig. 1: Antibacterial activities profile (inhibitory zone in diameter) of ethanol and methanol extracts at 50 mg/ml against tested bacterial species. Values are mean of three replicates

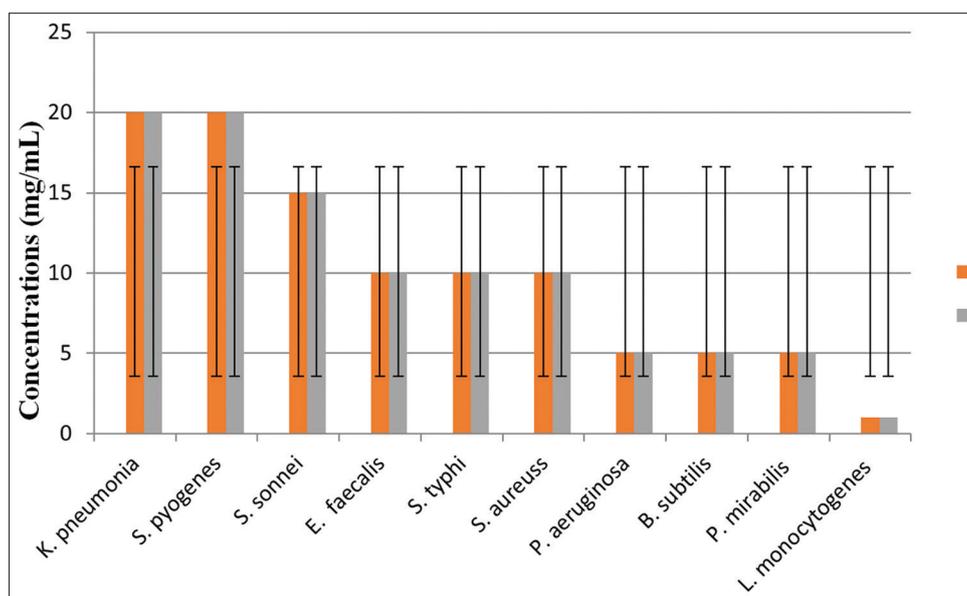


Fig. 2: Minimum inhibitory concentration values of methanol and ethanol leaf extracts with standard deviation against tested bacteria

Table 2: *In vitro* microscopic cell-based cytotoxicity assay (Vero cell line)

Extracts	Concentrations (µg/ml)										
	2000	1000	500	250	125	64	32	16	8	4	0
Methanol and ethanol	+	±	-	-	-	-	-	-	-	-	-

+: Positive (morphological alterations, CPE forming), -: Negative (No morphological alterations no CPE), CPE: Cytopathic effect

treated with *T. aphylla* leaves extracts (4 and 2500 µg/ml), versus untreated cells. The maximum concentration of methanol, ethanol, and water extracts from *T. aphylla* used in cell viability assay was 2500 µg/ml, and the result is presented in Table 2. This result incited us to look for another *in vitro* test to study the effect of extracts on MCF-7 cancer cell viability.

**Inhibitory effects of *T. aphylla* leaf extracts on breast adenocarcinoma cells**

The antiproliferative effect of both ethanol and methanol extracts of *T. aphylla* leaves were investigated against MCF-7 (Fig. 3). All methanolic and ethanolic extract inhibited MCF-7 cancer cells in a dose-dependent manner. At the lower concentrations (4–16 µg/ml), statistically no significant decrease of cell growth was observed. However, at the concentrations of 500–1000 µg/ml, the plant extract significantly inhibited the growth of MCF-7 cells as compared to the control ones (Fig. 3). For instance, 500 µg/ml ethanol and methanol extracts inhibited the cell growth by 55% (Fig. 3).

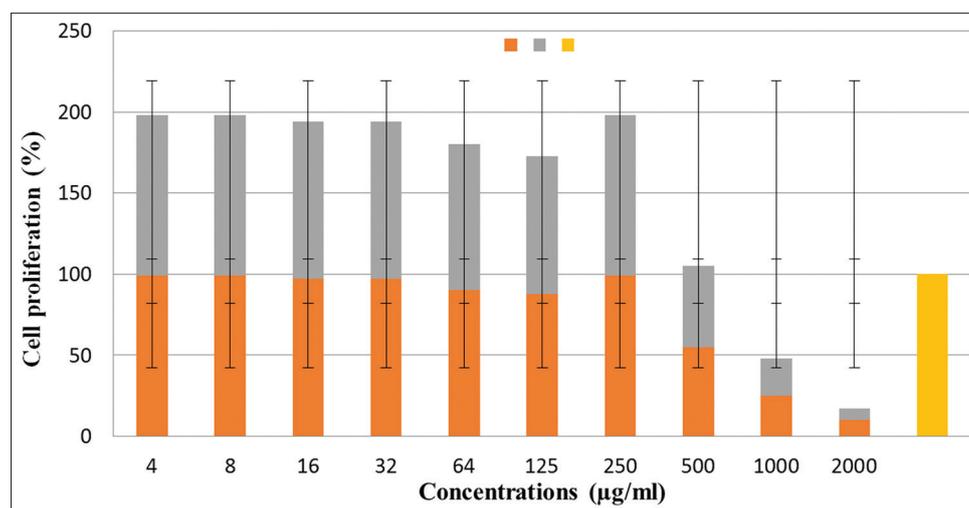
**DISCUSSION**

The major challenge facing the medical and public community is the shortage of antimicrobial compounds. This has arisen due to the overprescribing of unnecessary antibiotics and other antimicrobials; as a consequence of bacterial resistance, arising to antimicrobial compound [25], [26]. Medicinal plants remain a major of potentially useful structures for the development of new chemotherapeutic agents [17]. The first step toward this purpose is the *in vitro* cytotoxicity, anticancer, and antibacterial activity assessment. Many studies have been screened antiviral, antibacterial, antifungal, anthelmintic, and anti-inflammatory properties of plants and whether they contain compounds with therapeutic activity or not [25-29]. Some of these observations have helped in identifying the active principle responsible for such activities, and in the developing drugs for the therapeutic use in human beings, antimicrobials of plant origin are effective in the treatment of several infections [27].

Recent scientific research has shown that many plants used in traditional medicine are potentially toxic, allergic, mutagenic, and/or carcinogenic [30,19]. Therefore, *in vitro* cytotoxic evaluation studies are important to obtain the effective safe new agents that have certain desirable properties such as little or no toxic effects on normal cells, high efficacy on multiple sites, capability of oral consumption, known mechanism of action, low cost, and acceptance in the community [32]. Cytotoxicity testing of medicinal plants is not only important to evaluate and validate the safety of medicinal plants for traditional use but also provides guidance in the search for new active compounds.

In the present study, the cytotoxic effect of the methanol and ethanol extracts of the *Tamarix* were investigated *in vitro* on Vero cells using CTB reagent. The cytotoxicity was evaluated on Vero cells in a dose-dependent manner at the end of 72 h incubation. The cytotoxicity indices low degrees of cellular degeneration at high concentration. In this regard, it is worthy to mention that the mean CC<sub>50</sub> value of methanol and ethanol extracts in this study was lower than 1000 µg/ml (Table 2) (Fig. 3). *In vitro* cytotoxicity results indicated that the *T. aphylla* could not be belonging to the toxic plant. Therefore, the results obtained in this study supported that the *T. aphylla* might be considered as pro-missing candidates for further evaluation against different kinds of other cell types both *in vitro* and *in vivo*.

The antiproliferative effect of *T. aphylla* leaves was investigated against MCF-7 cells. The Vero cells were used for comparisons between normal and cancer cells in this study, and the result showed that the extract does not show any apparent cytotoxicity against the normal cells. Results indicated that the *T. aphylla* leaf extracts inhibited the proliferation of MCF-7 cells in a dose-dependent manner (Fig. 3) with the significant cytotoxic effect at 24 h with a concentration of 50 mg/mL. MCF-7 cells in this study were exhibited anticancer activities in the concentration-dependent manner at 500 and 1000 µg/ml. This is in agreement with other studies on the interesting anticancer activity of *Tamarix* species [24,31,32]. For instance, *Tamarix aphylla* leaves showed



**Fig. 3: Measurements of cell proliferation using Cell Titer-Blue assay in MCF-7 cancer cell line treated with different concentrations of *Tamarix aphylla* leaf extracts. Cells at  $2 \times 10^4$  cells/ml were cultured in the absence (control/phosphate-buffered saline or Dulbecco's Modified Eagle's Medium) or in the presence of *T. aphylla* for 72 h. Values represent the means of three independent experiments  $\pm$  standard deviation**

anticancer capacity against human leukemia and squamous carcinoma cells. Medicinal plant possesses that anticancer activity is regarded as one of the most attractives to explore natural anticancer agent for cancer control since certain natural compounds present in medicinal plants have efficiently inhibited tumor development in various human organs [33,34]. Another study showed that methyl ferulate from *Tamarix aucheriana* inhibits growth and enhances chemosensitivity of human colorectal cancer cells [12]. Antiproliferative of *T. aphylla* leaf extracts showed better cytotoxicity activity against MCF-7 cells, and therefore, it will be used for further antiproliferative investigation.

The antibacterial activity of *T. aphylla* has been evaluated *in vitro* against 10 pathogenic bacteria (Table 1). Several studies have been shown that *T. aphylla* has antibacterial, antifungal, antiviral, and anticancer activities [13,31,35,36]. All extracts tested showed antibacterial activity with variable inhibition effects and differences in their activities against pathogenic bacteria ranging from very strong inhibition to low. However, the extracts differ in their activities against the microorganisms tested, and an increase in the extract concentration from 50 to 100 mg resulted in a significant increase in the diameter zone of inhibition formed against all tested bacterium (Figs. 1 and 2). Our finding differs from previous studies that noted alcohols to be reliable and consistent solvents for the extraction of antimicrobial substances from medicinal plants [39]. This may be described by the fact that the secondary metabolites responsible for demonstrating antibacterial activity are greatly dependent on solvent system and collection process of metabolites from the plant sources [39]. Moreover, the geographical area and environment also affect the chemical composition of the plants and lead to the variation in activity [37,38]. The mean zone of inhibition produced by the methanol and ethanol extracts from *T. aphylla* leaves against *L. monocytogenes* was low (Fig. 2), and it is generally expected that a greater number of compounds would be active against Gram-negative rather than Gram-positive bacteria [41]. In a research conducted by Joshi *et al.* [41] using aqueous and methanolic extract of leaves of *A. hierochuntica* indicated that this plant showed better antibacterial activity effects against both Gram-positive and negative bacteria, except for *L. monocytogenes*. These results are quite similar to that of our present study although the sample preparation and some organisms were different.

#### CONCLUSIONS

This study demonstrated that the *T. aphylla* leaf extracts showed lower toxicity on normal cell line (low toxic plant) and significantly inhibited the growth of cancer cells. The *in vitro* antibacterial bioassay results of

this study showed that *T. aphylla* plant possesses potential antibacterial biomolecules against multidrug-resistant human pathogens and appeared to be a possible candidate for further pharmacological, phytochemical, and chromatographic studies to isolate and identify the bioactive compounds.

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#### AUTHORS' CONTRIBUTION

The present study was designed and all *in vitro* experiments were performed by the author himself.

#### CONFLICTS OF INTEREST

The author declares that he has no conflict of interest. The present research work was conducted by following the official protocols, and there are no financial involvements of any type, with any person or organization in this regard.

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