Research Article

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Evaluation of antibiofilm and cytotoxicity effect of Rumex vesicarius methanol extract

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Abstract
Background – Bacterial resistant to antibiotics represents an obstacle in medication management in hospitals. Biofilm can be easily formed by bacteria in indwelling medical devices. By increasing numbers of patients using indwelling medical devices, we have to find an effective antibiofilm for the eradication of biofilm-associated infections.

Methods – The present study was designed to evaluate the antibiofilm and cytotoxicity effect of methanol extract of Rumex vesicarius. Antibacterial and antibiofilm assays were investigated in this study against different standard and pathogenic bacteria isolates from endotracheal tubes in intensive care units (Staphylococcus aureus, Staphylococcus epidermidis, Proteus vulgaris, Klebsiella pneumoniae, and Pseudomonas aeruginosus). Scanning electron microscopy was used to demonstrate the reduction of biofilm formation using methanol extract of R. vesicarius. Also, cytotoxicity of R. vesicarius L. was evaluated by using the lactate dehydrogenase assay.

Results – R. vesicarius displayed a broad spectrum and antibacterial activity against the tested organisms. The minimal inhibitory concentration of the methanol extract was 62.5–125 mg/mL for gram positive while in case of gram negative, it was 125–250 mg/mL. While the result in case of minimal bactericidal concentration was 250–500 mg/mL in case of gram positive and was 500–1,000 mg/mL in case of gram negative.

Conclusion – Our results recommend usage of R. vesicarius as a promising antibiofilm to combat infection in indwelling medical devices.

Keywords: biofilm, endotracheal, Pseudomonas aeruginosus, Rumex vesicarius

1 Introduction

Biofilm exhibits a high affinity to attach to living tissues and indwelling medical devices. Biofilm-associated cells represent a major problem in antibiotics resistance as bacteria inside exopolysaccharides matrix of biofilm are shielded from both antibiotics and host immune system [1]. Most bacteria are able to grow as planktonic and biofilm forms. Simply biofilm is a single or multiple species that are embedded in self-produced extracellular polymeric substances. Basically, bacterial infections are frequently associated with biofilm [2].

Indwelling medical devices in the intensive care units cause high risk of infections and represent ideal surfaces for a biofilm attachment. Ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections are from the most important causes of nosocomial infections. Also, the use of different kinds of catheters, endotracheal tubes (ETT), and indwelling devices represent the main causes for the transmission of nosocomial infections [3]. Bacterial biofilm is one of the main causes of respiratory infections in most cases with ETT. Recently, the connection between VAP and biofilm has been well known, as pneumonia is related to the existence of biofilm rather than period of intubation [4,5].

Biofilms established on living and nonliving surfaces are not easily eradicated, basically because sessile cells that grown from biofilm are more resistance to antimicrobial agents or biocides by 10–1,000 fold [6,7].
Biofilms adversely affect a lot of human activities, so many trials have been done to prevent and eradicate biofilm. These trials can be succeeded by realizing the mechanism of biofilm consistency and assisting the critical contributions of biofilm [8,9]. As stated by NIH and CDC, more than 60% of hospital acquired infections are associated with biofilm formation [10].

As the role of biofilms in pathogenesis and drug resistance has been investigated, various strategies have been developed to eradicate and disrupt biofilm [11]. Antibiofilm agents with broad-spectrum action and safety effect could be a perfect target for drug development to eradicate infection caused by majority of biofilm-forming microbes.

Extractions from plants have been investigated for possible applications as antibiotic or antibiofilm. Even though a lot of researches on plants and their active constituents are being under investigation, the focus is mainly on their antimicrobial properties against bacteria not biofilms, so more investigations are needed concerning about eradication of resistant biofilm [12].

In Saudi Arabia and most Arabic countries, Rumex vesicarius, named hummayed or Hammad, is a terrestrial foliate plant corresponding to the family of Polygonaceae containing about 150 species found all over the world. The main chemical constituents of Rumex are anthraquinones and flavonoids [13]. R. vesicarius L. is used as a sorrel and collected in spring time and eaten fresh or cooked. The plant has been used for the treatment of some type’s tumors, hepatic diseases, bad digestion [14].

Previous studies revealed that genus Rumex exhibited antibacterial, bacteriostatic, and antiviral activities [15]. The effect of plants is due to bioactive constituents they had. It is always thought that medicinal plants have less side effect than chemotherapeutic drugs [16]. Therefore, natural plants are a promising novel antibacterial and antibiofilm. However, many reviews showed that many extracts of plants produce adverse effects [17], even some showed toxic effects [18]. So, we need to evaluate the toxic effect of medicinal plants before we use them as antibacterial or antibiofilm.

The gas chromatography–mass spectrometry (GC–MS) analysis of the R. vesicarius L. extracts revealed that active ingredients of plant consists of phytocomponents; totally, 211 constituents were detected by analysis extracts of the plant with different solvents. Hexane extract showed the highest number of 61 phytoconstituents, while in ethanol extract, the lower number of 45 phytoconstituents was detected which including both major and minor constituents [19].

Also, HPLC of a study detected the presence of p-coumaric acid, ferulic acid, chromone, catechin, and emodin [20].

The present work was aimed to screen the antibiofilm effect of methanolic extract of leaves from R. vesicarius extract against biofilm formed by different types of bacteria isolated from ETTs and, also, assess the cytotoxic effect of R. vesicarius extract. To our knowledge, the antibiofilm effect of R. vesicarius remains uninvestigated. Thus, the present study is the first to evaluate the antibiofilm effect of R. vesicarius extract against some gram-positive and gram-negative isolates.

2 Methods

2.1 Isolation of pathogens

Specimens were collected from endotracheal aspirates of mechanically ventilated patients in respiratory intensive care unit, Zagazig University hospitals, Egypt, from July 2017 till September 2017. Samples were inserted in a sterile tube and transferred to the laboratory where incubated for 24 h in 37°C. The broth was first checked for the presence of bacteria growth by a direct Gram stain smear. Subculture was made for positive samples on chocolate, MacConkey agar, and blood agar and incubated in 37°C for 24–48 h, then, the macroscopic and microscopic examination was determined, and based on their morphology, standard identification biochemical tests for gram negative and gram positive were performed.

2.2 Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed to isolate resistant bacteria by disk diffusion method as per Clinical and Laboratory Standards Institute.

2.3 Plant material

Plants of R. vesicarius L. were collected freshly from Al-Qassim region, Kingdom of Saudi Arabia during 2017 at the flowering stage. The collected plants were carefully examined and identified by Dr. Rizwana Humaria King Saud University, Department of Botany and Microbiology, College of science, by the aid of regional floras [21,22]. The leaves were air dried, powdered, and then stored in an air tight container for further analysis.
2.4 Extraction

Twenty grams of dry leaves of *R. vesicarius* was grinded into fine powder, then extracted by decoction by boiling with methanol for 5 min, followed by filtration and concentration of the filtrate, and evaporated till dryness on water bath to afford one gram extract. For further use, the extract was stored in an airtight container at 4°C in the refrigerator.

2.5 Detection of unsaturated fatty acids – Bayer’s test

Potassium permanganate 1% is used for the qualitative detection of unsaturated fatty acids [23].

2.6 Determination of total phenolic content

The total phenolic contents of *R. vesicarius* leaves were estimated according to ref. [24]. The data were detected in milligrams of Gallic acid per gram of dry weight of plant extract (mg GAE/g DW). The data were triple checked and were represented as mean ± standard deviation (SD).

2.7 Detection of the presence of steroids and terpenoids

0.5 mL of anhydrous acetic acid was added with 2 drops of conc. H$_2$SO$_4$ to 1 mL of *R. vesicarius* [25].

2.8 Determination of flavonoids

The colorimetric method assay was used to quantify the flavonoids content of extract according to [26]. The data were detected as mg catechin equivalents per gram of dry weight (mg CE/g DW).

2.9 Antibacterial activity

2.9.1 Agar well diffusion

The antibacterial effect of leaves extract was evaluated against *Staphylococcus aureus* (ATCC: 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC: 9027), *Proteus vulgaris* (ATCC 6380), *Klebsiella pneumoniae* (ATCC700603), and pathogenic isolates. The well diffusion method was used to determine the antibacterial activity of extracts of *R. vesicarius*. Different concentrations of extract from 500 to 31.25 mg/mL were adjusted by dissolving in dimethyl sulfoxide (DMSO). Muller Hinton agar was plated with bacterial isolates, and then, 100 μL of different concentration of extracts was poured in well. 5% DMSO was used as negative control while the positive control was Ciprofloxacin solution 10 μL/mL. All plates were incubated at 37°C for 24 h, and then, the plates were detected for the zone of inhibition around the wells.

2.9.2 Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

To determine MIC and MBC, the broth micro-dilution method was used with different concentrations of *R. vesicarius* L. extract. The 96-well microtiter plates were filled with 100 μL of bacteria about 10$^8$ CFU/mL and then 100 μL of different concentrations of leaves extract (500, 250, 125, 62.5, 31.25 mg/mL) was added in separate well. First, the absorbance of each well was detected by an automatic enzyme linked immunosorbent assay (ELISA) tray reader at 630 nm. Then, after 24 h incubation with agitation at 37°C, the absorbance was detected by ELISA at 630 nm. These absorbance values were subtracted from the results before incubation [27,28].

2.10 Biofilm inhibition assay quantitative and qualitative

96 well flat-bottom polystyrene plates were incubated with bacteria for 24 h and diluted 1:100 into 200 μL of trypticase soy broth and serial dilution of (3,000 mg) methanol extract of *R. vesicarius* L. at 37°C for 24 h was performed without agitation. In column 11, the negative control was done by adding 200 μL of sterile trypticase soy with 5% DMSO broth, and in column 12, positive control was done by adding 200 μL of bacteria suspension. After a further 24 h incubation, wells were washed triple with distilled water to remove planktonic bacteria then, biofilms were detected by staining with 0.05% crystal violet for 15 min before reading absorbance excess crystal violet was removed by washing with distilled water and crystal violet attached was solubilized in 200 μL of 95% methanol. Biofilms were quantified by reading the microplates at 570 nm (Biotek Elx800, USA) [29].
To qualitatively assay of biofilm inhibition, scanning electron microscopy (SEM) was detected by establishing biofilm of St. epidermidis in 96-well, treated with DMSO and extract of R. vesicarius (2,000 mg). Biofilms on the wells were washed with PBS and dried at 60°C. For SEM, biofilms were fixed with 2.5% glutaraldehyde and dehydrated with graded ethanol. Biofilms were freeze-dried, gold-coated, and subjected to SEM.

### 2.11 Cytotoxicity effect

To know the cytotoxicity effect of R. vesicarius extract in vivo, Madin Darby canine kidney (MDCK) tissue cells were used as a model for mammalian epithelial and the method was carried out as following:

1. MDCK tissue cells were grown to ~90% confluence in a 24-well sterile Corning plate.
2. Then, we added 100 µL of R. vesicarius extract, 100 µL of DMSO, and 100 µL of bacteria St. aureus as positive control.
3. The cells were observed under a microscope to determine when epithelium has 75% confluence and tight junctions between cells are broken down at 8, 16, and 24 h.
4. We used lactate dehydrogenase (LDH) assay which is used to test tissue cell toxicity. Briefly, it is a test for cell death by assessing the plasma membrane damage. If the cell is damaged, it releases a product LDH, which can be measured using a colorimetric assay with a plate reader.

### 3 Results

#### 3.1 Isolation of pathogens

The study was carried out on 75 patients on ventilator. Out of 75 specimens, 60 specimens were found to be positive in cultures with 86 isolates. According to microbiological results, Ps. aeruginosa was the prevailing bacteria with 30.23%, St. aureus (26.74%), K. pneumoniae (17.44%), St. epidermidis (18.6%), and P. vulgaris (6.9%); these results are represented in Figure 1.

#### 3.2 Antimicrobial susceptibility

The results revealed that Ps. aeruginosa was highly resistant to cefixime by (74–82%). While, susceptibility of gram positive was highly resistance to oxacillin (72.1–74.3%), cefatholin (65.3–66.7%), and ciprofloxacin (44.5–46.6%).

#### 3.3 Detection of unsaturated fatty acids – Bayer’s test

The disappearance of the characteristic purple colour of KMnO₄ and formation of brown Precipitate are a confirmatory test of presence of unsaturated fatty acids.

#### 3.4 Detection of the presence of steroids and terpenoids

Qualitative determination has been done by adding acetic anhydride and conc. H₂SO₄ to 1 mL of R. vesicarius extract appearing of blue color detect steroids while presence of ring with blue green color detect trepenoids.

#### 3.5 Determination of total phenolic content and flavonoids

The total phenolic content of R. vesicarius leaves extract was 58.55 ± 0.02 mg GE/g DW. The quantification of total flavonoids was 37.87 ± 0.02 mg CE/g DW.

![Figure 1: Percentage of isolated microorganisms from ventilation tubes.](image)
3.6 Antibacterial activity of *R. vesicarius*

Antibacterial activity of *R. vesicarius* was evaluated against a set of standard strains and clinically significant strains isolated from intensive care units (ICU) and potency were assessed by measuring the inhibition zones.

The antibacterial activity of *R. vesicarius* leaves extract is presented in Table 1. Antibacterial effect was directly proportional with an increase in concentration as methanol extract of *R. vesicarius* at 250 mg/mL had inhibition zone 17.60 ± 0.80 to 18.84 ± 1.11 and 15.90 ± 1.21 to 16.40 ± 1.11 for standard and pathogenic gram-positive isolates, respectively, while in case of gram negative, 17.50 ± 1.31 to 18.62 ± 1.21 and 15.90 ± 1.12 to 16.90 ± 0.91 for standard and pathogenic, respectively.

Also, the results revealed that extract of leaves of *R. vesicarius* displayed a broad spectrum and antibacterial activity against the tested organisms. The MIC of the methanol extract was 62.5 ± 1.11 for *P. vulgaris*, 125 mg/mL for gram positive isolates, respectively, while in case of gram negative, it was 125–250 mg/mL. The result in case of MBC was 250–500 mg/mL in case of gram positive and was 500–1,000 mg/mL in case of gram negative.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (mg/mL)</th>
<th>MBC (mg/mL)</th>
<th>Ciprofloxacin (10 μL/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>St. aureus</em> (ATCC: 29213)</td>
<td>125</td>
<td>250</td>
<td>20.10 ± 1.21</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (ATCC 700603)</td>
<td>500</td>
<td>1,000</td>
<td>22.40 ± 0.94</td>
</tr>
<tr>
<td><em>Ps. aeruginosa</em> (ATCC: 9027)</td>
<td>250</td>
<td>500</td>
<td>21.12 ± 0.81</td>
</tr>
</tbody>
</table>

3.7 Biofilm inhibition assay quantitative and qualitative

Quantitative assay of biofilm inhibition revealed that the percentage of reduction of biofilm formation treated with extract at concentrations 1,000 and 2,000 mg significantly reduced the biofilm of *St. aureus* by 57 and 66%, respectively, while in case of *St. epidermidis*, the percentage of reduction was 62–71%, respectively. In case of gram negative, the concentration used was 2,000 and 3,000 mg and the percentage of reduction in case of *Ps. aeruginosa* was 45 and 52%, respectively, while the percentage was 53.5 and 60% in case of *P. vulgaris*. The percentage with *K. pneumoniae* was 55.6 and 61%, respectively. The results are shown in Figure 2. A confirmatory result was also obtained with the SEM images as it showed disruption in the biofilms’ integrity and cells were scattered in the presence of extract of *R. vesicarius* L. as revealed in Figure 3.

3.8 Cytotoxicity of extract

From measuring of LHD assay in Figures 4–6, the results showed that the plant extract has a low cytotoxicity effect...
to epithelial cells of MDCK even after exposure to 24 h. Also, Figure 7(a)–(d) reveals that epithelial cells still alive under microscope even after incubation with *R. vesicarius* extract for 24 h.

4 Discussion

Indwelling medical devices including endotracheal intubation and mechanical ventilation are intensively used in

![Figure 2](image)

**Figure 2**: Effect of different concentrations of methanol extract of *R. vesicarius* L. on biofilm formation of different isolates.

![Figure 3](image)

**Figure 3**: Effect of methanol extract of *R. vesicarius* L. on biofilm formation. (a) SEM image of *St. epidermidis* biofilms treated with DMSO and (b) treated with *R. vesicarius* 2,000 mg.

![Figure 4](image)

**Figure 4**: The percentage of LDH release with plant extract, control, DMSO, and positive control after 8 h incubation with MDCK cells.
sepsis, acute respiratory disorders, and neurological dysfunctions. Ventilators are widely used to prevent deaths in patients with respiratory failure however. They represent good environments for biofilm formation, which represents a threat on life in immune-compromised patients. Actually, bacteria growing in biofilm showed high resistance to antimicrobial agents; consequently, infection will be intractable to cure.

According to the diagnosis of patients in the ICU, staying a long period in the hospital, and policies of antimicrobial agents, different organisms are isolated [30]. The ETTs change host defenses and obstacle mechanical clearance. Moreover, the patients in the respiratory intensive care unit are extremely sick and more vulnerable to acquire nosocomial infections [31]. ETTs deteriorate mucociliary function and expedite accumulation of bronchial secretions. Also, ETTs damage the mucosa and facilitate the way for biofilm formation and consequently contribute to multidrug resistance. Out of 75 samples submitted for microbial culture, 60 (80%) were found positive for microbial growth. This result was also reported by other studies [32–34]. In the present study, the most frequent isolates were Ps. aeruginosa 30.23%, St. aureus 26.74%, St. epidermidis 18.63%, K. pneumoniae 17.44%, and P. vulgaris 6.9%. Based on these results, Ps. aeruginosa was the widespread bacteria; this is in agreement with other studies [33,35].

Ps. aeruginosa is one of the most opportunistic bacteria and has a high incidence of nosocomial infections in ICU. The importance of Ps. aeruginosa is that it is naturally resistance to a wide range of antibiotics; consequently, it has the ability to infect critically ill patients associated with the high rate of morbidities and mortality [36,37]. Subsequently, in the present study, Ps. aeruginosa isolates were highly antibiotic resistant and, apart from other antibiotics, showed 75.2, 70.5, 67.8, and 62.1%
resistance to Cefixime, Ceftriaxone, Pipracillin, and Imipenem antibiotics respectively. This was higher than the rate reported by ref. [34]. On the other hand, *P. vulgaris* showed high resistance to ceftriaxone and piperacillin by 68.6 and 65.3%, respectively; these results were in agreement with other study [38]. The resistance pattern of gram positive ranged from 65.3 to 66.7% to cefatholin, 72.1 to 74.3% to oxacillin, and 44.5 to 46.6% to ciprofloxacin.

Biologically active constituents like phenolics and flavonoids are used as anticancer, antioxidant, and antimicrobial agents. The presence of phenolics and flavonoids in plants interferes with some essential biological pathway in microbial agents [39]. In this study, we extracted the active constituents from leaves using methanol as it gives more yield; this is in agreement with another study [40,41]. The *R. vesicarius* leaves were used as aerial parts of this plant displayed concentrated yields from all active constituents than root parts [42].

The total phenolic content of leaves of *R. vesicarius* was $58.55 \pm 0.02$ mg GE/g DW. The amount of phenolics per each extract concentration was expressed as gallic acid equivalent; this is in agreement with other study [43].

The quantification of total flavonoids was $37.87 \pm 0.02$ mg CE/g DW. A similar result was observed in the quantification of total flavonoids [44]. Also, a study of chemical constituents of *R. vesicarius* detected the presence of polyphenols, flavonoids, carotenoids, tocopherols, and ascorbic acid in different parts of *R. vesicarius* [45,46]. These results revealed that there is a relationship between polyphenolics and antimicrobial effect of plant, as it was...
reported that phenolic compounds, tannins, anthocyanin, and flavonoids have different mechanisms in free radical scavenging inhibition [46,47].

Recently, a study detected the chemical constituents of *R. vesicarius*; the main groups were fatty acids and their derivatives, oxygenated hydrocarbons, and high oleic acid were detected by 70–80% [46,48].

The results of the antibacterial effect of methanolic extracts of *R. vesicarius* L. in terms of zone of inhibition showed a moderate antibacterial activity against all five bacterial strains. Previous studies obtained similar results for the antibacterial activity of *R. vesicarius* L. extract and showed that *R. vesicarius* exhibits high antimicrobial activity against several bacteria [49–51]. Antibacterial potency has been attributed to terpenes, oxygenated hydrocarbons, and carbohydrate, which are chemical components of *R. vesicarius* [47,49,50].

Polyphenols contain a wide variety of polyphenol molecules structure, basically useful structures for the innovation of new antimicrobial agents [52]. Destabilization of lipopolysaccharide membrane by phenolic compounds reported in a study may be attributed to antimicrobial action of *R. vesicarius* against gram-negative bacteria [53]. Also, a study revealed that the GC–MS analysis of methanol extract of the plant has essential phytoconstituents such as 2-propyltetrahydro-2H-pyran-3-ol, ethyl 2-hydroxycyclohexane-1-carboxylate, 1,3-dihydroxypropan-2-yl olate, N2,N4-diisopropyl-6-(methylsulfonyl)-1,3,5-triazine-2,4-diamine, (E)-octadecl-13-enoic acid, 2-hydroxypropane-1,3-diyldipalmitate, high oleic safflower oil, 2,3-dihydroxypalmitoylethyle, methyl octadecl-8,11-diyanoate, (E)-(2-(chloroimino)-3-methylbutanoyl)-l-valine, methyl 5-((1R,2R)-2-undecyloxypropyl)-pentanoate, and (2-phenyl-1,3-dioxolan-4-yl)methyl olate [47]. Palmitic acid had effectively killed gram-negative bacteria as reported in a study [54]. A complete profile of bioactive compounds of *R. vesicarius* L. was previously investigated in a study which revealed that leaves of *R. vesicarius* were rich in vitamins especially vitamin C (1,330 mg/100 g), vitamin E (2371.7 mg/100 g dry wt), and β-carotene (252.7 µg/100 g dry wt) [45].

A recent study confirmed that *R. vesicarius* has flavonoids and phenolics compounds that exhibited antimicrobial activity against many resistance bacteria including gram-positive and -negative bacteria [48].

A study of the MIC of the plant extracts on the tested organisms has revealed that the extracts possess antimicrobial activity at various concentrations but highest at 250 mg/mL. The antibacterial effects of extracts on the tested organisms increase by increasing the concentrations. Similar result was also reported in a study [55].

Several previous experiments on different plant parts of different species of *Rumex* confirmed potent antibacterial activity against both gram-positive and gram-negative bacteria [56]. Also, a study to determine MIC of palmitic acid on some gram positive and gram negative revealed that more than 256 µg/mL was the MIC for both gram-negative and -positive bacteria [57].

Bacteria in sessile form showed highly persistence and resistance to antimicrobial agents, unlike those planktonic form. This may be attributed to bacteria in biofilm that are embedded in extra polysaccharide matrix representing a barrier that hinders diffusion of antimicrobial agents to colony [58].

Needing to develop new drugs opens the way to extract biologically active compounds from natural products. In this study, anti-biofilm effects of *R. vesicarius* L. had been significant. The results of this study showed that the methanol extract of *R. vesicarius* L. has antibiofilm activity on gram-positive and -negative isolates. The results showed that the methanol extract administrated significantly reduced the biofilm biomass and the increase in the methanol extract concentration decreased the biofilm formation. It could be possible that the anti-biofilm activity could be due to interfering with the extracellular polymeric production of cells [57,59]. Also, the antibiofilm effect could be due to the presence of flavonoids, anthraquinones, carotenoids, and organic acids [49]. Also, a recent study confirmed the presence of β-sitosterol [43] and this compound reduced quorum sensing in *Ps. aeruginosa* [60].

Emodin, a constituent of *R. vesicarius* extract, reduced the biofilm formation of *St. aureus* [61] and *Ps. Aeruginosa* [62].

A study reported that unsaturated fatty acids at sub-MIC inhibited the biofilm formation of *St. aureus* [63].

To estimate the cytotoxicity of *R. vesicarius* L. extract to epithelia cells, we used LDH assay. The basis of this assay depends on the spontaneous release of LDH, upon disruption of the cell membrane. The results obtained indicate very low toxicity to *R. vesicarius* L. extract and these results were supported by seeing MDCK cells under microscope and showing viability of epithelial cells after 24 h treating with plant extract. Even the low cytotoxicity may be attributed to DMSO used for dissolving extract so we can further try another substance with no toxicity on cells to dissolve extract. These results in agreement with El-Hawary who reported that *R. vesicarius* has antioxidiant, protective effect on liver when used for the intoxication of CCl4 in rats [45]. A recent study revealed that *R. vesicarius* extract was unharmed at 6,000 mg/kg [21].
Our results suggested that the extract has a beneficial effect on biofilm formation reduction. Similar results were found with methanol extract Rumex dentatus against biofilm formation of Ps. aeruginosa [63].

To our knowledge, this is the first study on the antibiofilm effect of R. vesicarius till now, and from the cytotoxicity results, we can recommend R. vesicarius L. as a promising antibiofilm treatment for patients in intensive care units and using indwelling medical devices, which support the growth of bacterial biofilm upon long-term usage.

5 Conclusions

There is an urgent need to study antibiofilm effect rather than antimicrobial effect, due to the serious problems that the biofilms have caused in hospitals and environmental and industrial contexts. The plant extract of R. vesicarius evaluated in the present research had potential antimicrobial and antibiofilm activities against gram-negative and -positive bacteria, which can be an alternative to control biofilms’ formation or can be used as model to the search for new drugs.

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Conflict of interest: The authors declare no conflict of interest.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

Data availability statement: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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