CHARACTERIZATION OF THE RISK FACTORS ASSOCIATED WITH URINARY TRACT INFECTION (UTI) IN LEBANON, AND EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF HAWTHORN EXTRACT AGAINST UTI-CAUSATIVE BACTERIA

Jamila Ramadan*, Rana El Hajj, Mahmoud Khalil

Address(es):
Department of Biological Sciences, Faculty of Sciences, Beirut Arab University, Debbiye Campus, Lebanon.

*Corresponding author: jkr46@student.bau.edu.lb

ABSTRACT

Urinary tract infections (UTIs) are a major cause of comorbidity in patients with implied conditions. Identifying the risk factors associated with UTI is crucial to control the severe outcome. In this study, we aimed to determine the factors associated with UTI, and identifying UTI most causative pathogens. In addition, we aimed at assessing the sensitivity of antibiotics on the most common strains causing UTI, and evaluating the antibacterial activity of the natural hawthorn extract in vitro. A total of 100 urine samples were collected from patients in Mount and South Lebanon. UTI causative strains were identified using Vitek 2 and antimicrobial susceptibility testing. The antibacterial activity of hawthorn was determined by agar well diffusion method, and biofilm formation assay. The results revealed a prevalence of 51% of UTI among patients in Mount and South Lebanon. UTI significantly correlated with different risk factors such as diabetes, heart disease, catheters use, caffeinated energy drinks, vitamin D deficiency, and menopause. Moreover, Hawthorn fruits and leaves ethanolic extract exhibited a good antibacterial activity against a wide range of Gram-positive and Gram-negative resistant bacteria. Thus, Hawthorn extract could serve as a promising therapeutic alternative for antibiotics used against UTI-causing bacteria.

Keywords: Urinary Tract Infections, Hawthorn extract, Uropathogens, risk factors, anti-bacterial activity

INTRODUCTION

Urinary tract infection (UTI) is the most common bacterial infection affecting public health. This infection is highly prevalent among Lebanese population, with an increased morbidity in the community (6). UTI symptoms mostly include burning pain, often associated with bleeding, and sepsis (11). Most of UTI infections are caused by gram-negative bacteria like K. pneumonia, E. coli, P. aeruginosa, P. vulgaris, P. mirabilis and other gram-positive bacteria like S. aureus, S. epidermidis and S. agalactiae (6). UTI begins when the periurethral is contaminated with pathogens from the gut. These pathogens colonize the urethra and migrate into the bladder. This colonization is mediated by specific bacterial structures involving pili for gram negative and adhesins for gram positive (6). Following infection, an inflammatory response characterized by neutrophil infiltration is stimulated. Some bacteria elude the immune system, either by invading host cells or by morphological changes resulting in neutrophil resistance, favoring bacterial multiplication and biofilm formation (6). Bacterial toxins and proteases cause host cell destruction, releasing vital nutrients for bacterial survival, and stimulating the ascension to the kidneys. Colonization of the kidneys causes tissue damage in the host. If still untreated, it can progress to bacteremia (6). In addition, UTI is associated with many risk factors related to medical conditions like diabetes, hypertension, heart failure, thyroid disease, obesity and sexual intercourse (6). More particularly, postmenopausal women may have higher risk of UTI than premenopausal women (9). Moreover, catheter use increases some bacterial infections in the urinary tract infection, such as E. coli (6). Also, vitamin D deficiency is associated with UTI since vitamin D plays important role in the regulation of immune system (17).

Several antibiotics are used in treatment of UTI, but the most appropriate ones depend on the type of bacteria found in urinary tract and on the complicated nature of infection. These antibiotics interfere with bacterial growth and block their multiplication (11). However, bacterial resistance is one of the major problems that limit the effectiveness of most of the commercialized antibiotics used for UTI treatment (21). Thus, the emergence for testing new compounds against bacteria causing UTI is still arising. Hawthorn extracts lately gained attention as therapeutic agents for many diseases. These extracts exhibited notable effects in promoting digestion, improving immune system, and reducing inflammation and bleeding (6). The present study evaluates the correlation between UTI and different health and lifestyle parameters among Lebanese women. It also investigates the activity of hawthorn extract on UTI causative bacteria. Our findings highlight on the importance of the medical use of natural products, which could serve as an alternative for antibiotics, particularly in limiting bacterial resistance.

MATERIAL AND METHODS

Sample collection

A total of 100 urine samples from women were collected from patients in Mount and South Lebanon. Patient’s demographics were recorded (age.). Additional information related to marital status, food and drink habits, and certain types of diseases were collected. Midstream urine samples were collected and processed at the time of collection.

Macroscopic and microscopic examination of urine samples

Macroscopic analysis was performed using coombe 10 in order to determine leucocyte esterase and nitrite. Urine samples were then centrifuged and examined microscopically. Aggregated white blood cells, presence of numerous red blood cells, epithelial cells, casts and crystals as well as bacteria and yeasts were indicative of UTI.

Isolation and Identification of microorganisms from urine samples

Urine samples with detected leucocyte and nitrite were cultured on blood agar, MacConkey’s agar, and Uri select agar for 24-48 hours. Detection of organisms is based on colonies pigmentation, hemolytic reaction on blood agar, and lactose fermentation on MacConkey’s agar

Identification of isolates

All bacterial isolates were identified by VITEK®2 system. Bacterial suspension was prepared by inoculating bacteria in 0.9% sodium chloride, and a suspension of 0.5 McFarland was prepared.

Antibacterial susceptibility testing

Bacterial isolates were swabbed on Muller-Hinton agar media then incubated for 24 hours at 37 °C. Antibiotics tested were Amoxicillin, Ciprofloxacin, Penicillin, Tetracycline, Norfloxacin, Imipenem, Nitrofurantoin, Posomycin, Augmentin, Cefotaxime, Vancomycin, Cephalothin, Nalidixic acid, Oxacillin, Aztreonam, Cefixime, Lincomycin, and Amikacin.
Collection and sampling of Hawthorn extracts
Fruits and leaves of hawthorn were collected from Chamis and Hasroun in Mount Lebanon during summer-autumn 2020.

Preparation of Hawthorn extract

Water and Ethanol Extract: Fruits and leaves of hawthorn were air-dried for 48 hours separately, then ground to a fine particle with mortar and pestle. Aqueous and ethanolic extracts were prepared by dissolving 10 grams of powdered fruits and leaves with 100 ml distilled water and 70% ethanol respectively. Thereafter, samples were filtered, lyophilized and the aqueous and ethanolic extract were collected.

Phytochemical Screening

Detection of effective compounds found in Hawthorn Extract Fruits and leaves
A phytochemical screening of Hawthorn extracts was performed. The extracts were screened for saponins, flavonoids, polyphenols, quinines, terpenoids, alkaloids, cardiac glycosides, tannins and steroids.

Saponins. Plant extract was mixed with distilled water and shaken vigorously. The persistence of froth formation indicated the presence of saponin.

Flavonoids. Plant extract is added to ethyl acetate then heated on water bath. Once cooled, the mixture was filtered, then shaken with dilute ammonia solution. A separation layer occurs and yellow color in the lower layer of ammonia indicates the presence of flavonoids.

Polyphenols. FeCl₃ (1%) and K₃[Fe(CN)₆] (1%) are added to plant extract. Fresh reddish blue color indicated the presence of polyphenols.

Terpenoids. Chloroform is added to extracts, followed by sulfuric acid. A reddish-brown color indicated the presence of terpenoids.

Alkaloids. 1% of HCl was mixed with extracts. Few drops of Mayer’s reagent added to the filtrate. Turbidity is a sign of positive detection.

Quinines. Concentrated sulfuric acid added to extracts. Presence of quinines is detected by formation of red color.

Tannins. Extract mixed with bromine water. The presence of tannins is detected by the decoloration of bromine water.

Steroids. Extracts are mixed with chloroform and concentrated sulfuric acid. The formation of red color in the lower layer of chloroform indicated the presence of steroids.

Cardiac Glycosides. Glacial acetic acid with ferric chloride solution are added to the extract. The formation of brown ring indicated that there is detection of deoxyxugar of cadenaolides. A greenish ring appears in the layer of acetic acid above the brown ring and violet ring below the brown ring are indicative of glycosides presence.

Antibiofilm inhibition

Prevention of initial bacterial cell attachment
A concentration of 400 mg/mL of hawthorn extract was added to bacterial pathogens and adjusted to 0.5 McFarland. Tetracycline was used as positive control while ethanol and distilled water were used a negative control separately. A crystal violet staining was performed and biofilm is detected appeared as a purple ring. Absorbance was measured at 630 nm using ELISA reader and the percentage of biofilm inhibition was determined by:

\[
\text{% inhibition} = \frac{OD_{\text{negative control}} - OD_{\text{experimental}}}{OD_{\text{negative control}}} \times 100
\]

Antibacterial activity

Agar Well diffusion method
Bacterial suspension was prepared with a density adjusted to 0.5 McFarland standard turbidity (1.5x10⁸ CFU/ml). Bacterial inoculum was swabbed and a hole of 6 - 8 mm in diameter is aseptically punched. A concentration of 400 mg/ml of ethanolic and aqueous extracts were added accordingly and incubated at 4 °C for 2 hours. Water and ethanol were used as negative control and tetracycline was used as a positive control. After incubation, the diameter of the zone of inhibition was measured in millimeters (mm).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Hawthorn extract
A stock solution of plant extract with a concentration of 400 mg/ml was prepared. Serial dilutions of Hawthorn extract were prepared and incubated for 24 hours with bacterial suspension. All tests are performed in triplicate.

Statistical analysis:
All the data were processed using SPSS software and expressed as mean ± SD.

RESULTS

Sampling Design
All participants of this study were females aged from 20 to 79 years old. The mean of the study was 40 ± 16.9 years.

Macroscopic analysis of urine samples
Macroscopic analysis showed that 57 % (N=57) cases of samples were positive for leukocytosis and pyuria. 51% (N=51) cases of samples were positive for bacteriuria and 24% (N=24) cases of samples were positive for nitrites.

Phenotypic Characterization of isolates
Overall, 17% (N=17) bacterial isolates were identified as Gram-positive cocci, S. aureus, S. epidermidis, Enterococcus. All isolates did not appear on MacConkey agar, and grown on blood agar and Uriselect agar. S. aureus formed grape-like, yellow colonies, and was identified as catalase-positive, oxidase negative, coagulase-positive. This strain also fermented mannitol and displayed a yellow color on mannitol agar and beta-hemolytic on blood agar.

However, S. epidermis appeared as white colonies on blood agar. It was identified as catalase-positive, coagulase-negative, and novobiocin sensitive, while Enterococcus appeared as small gray colonies and gamma hemolytic on blood agar, and was identified as catalase-negative.

Moreover, 34% of bacterial isolates (N=34) showed Gram-negative bacilli and grew on MacConkey’s agar as pink colonies. There was no hemolysis on blood agar and each microorganism developed its specific characteristics on Uriselect agar.

Antibiotic Sensitivity tests against different identified bacterial isolates
Different antibiotics were test against gram positive and gram negative UTI-causing bacteria as shown in table 1. Bacteria showing a high resistance rate were selected for further testing.
### Table 1 Classification of identified bacterial isolates based on the resistance, sensitivity or intermediate effect of different antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic agent</th>
<th>Bacteria</th>
<th>% if Sensitive</th>
<th>% if Resistant</th>
<th>% if Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IMIPENEM (IMP)</strong></td>
<td>S. aureus</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>83</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td><strong>NITROFURANTOIN(FTN)</strong></td>
<td>S. aureus</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>40</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>29</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>83</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td><strong>CEFUROXIME(CXM)</strong></td>
<td>S. aureus</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>20</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>10</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>35</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>33</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td><strong>CEFOTAXIME(CTX)</strong></td>
<td>S. aureus</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>86</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>37</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td><strong>CIPROFLOXACIN(CIP)</strong></td>
<td>S. aureus</td>
<td>60</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>71</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>58</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td><strong>AUGMENTIN(AMC)</strong></td>
<td>S. aureus</td>
<td>60</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>71</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>54</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td><strong>CEFIXIME(FIX)</strong></td>
<td>S. aureus</td>
<td>60</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>40</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>57</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>42</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td><strong>COLISTIN (COL)</strong></td>
<td>S. aureus</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>60</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>20</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>71</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td><strong>FOSFOMYCIN(FOS)</strong></td>
<td>S. aureus</td>
<td>40</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>43</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S. marcescens</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

**Identification of uropathogens in collected UTI Samples:**

A total of 51% of urine samples showed significant bacteriuria (N=51). The most prevalent uropathogens were *E. coli* 47% (N=24) followed by *K. pneumoniae* 15.7%, (N=8), *S. epidermidis* 13.8% (N=7), *aureus* 9.8% (N=5), *Enterococcus* 9.8% (N=5) and *Serratia* 3.9% (N=2) (Figure 1).
Correlation between UTI, age and body weight of UTI patients

UTI was mostly predominant in women with menopause, with age ranging from 45 to 79 years. UTI causing bacteria was most predominantly found in 67% of obese patients, with a body weight ranging between 61 to 104 kg (N=34) (Table 3).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>(+) UTI</th>
<th>(-) UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 44</td>
<td>N=25 (49%)</td>
<td>N=40 (82%)</td>
</tr>
<tr>
<td>45 – 79</td>
<td>N=26 (51%)</td>
<td>N=9 (18 %)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body Weight (kg)</th>
<th>(+) UTI</th>
<th>(-) UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 – 60</td>
<td>17(33%)</td>
<td>36(73%)</td>
</tr>
<tr>
<td>61 – 104</td>
<td>34(67%)</td>
<td>13(27%)</td>
</tr>
</tbody>
</table>

Correlation between UTI, age and body weight of UTI patients

The risk factors associated with UTI are evaluated and represented in table 3. Among which, menopausal women, marital status, a previous history of UTI, heart disease, diabetes, coffee, energy drinks, catheter use, vitamin D deficiency significantly correlated with this infection.

Table 3 Correlation between UTI and sociodemographic variables, health condition, and food habits variables. CI: Confidence Interval, P: Probability; p ≤ 0.05 value is statistically significant.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Odds Ratio</th>
<th>95 % CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital Status</td>
<td>4.335</td>
<td>1.553 – 12.210</td>
<td>0.003</td>
</tr>
<tr>
<td>History of UTI</td>
<td>2.455</td>
<td>1.092 - 5.517</td>
<td>0.028</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.743</td>
<td>1.014 – 7.419</td>
<td>0.042</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.846</td>
<td>0.660 – 5.166</td>
<td>0.239</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>0.120</td>
<td>0.014 – 1.015</td>
<td>0.023</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.333</td>
<td>0.427 – 4.168</td>
<td>0.620</td>
</tr>
<tr>
<td>Thyroid Disease</td>
<td>0.225</td>
<td>0.024 – 2.088</td>
<td>0.155</td>
</tr>
<tr>
<td>Contraception</td>
<td>0.483</td>
<td>0.150 – 1.560</td>
<td>0.217</td>
</tr>
<tr>
<td>Catheter</td>
<td>6.400</td>
<td>0.741 – 55.258</td>
<td>0.050</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.950</td>
<td>0.367 – 2.447</td>
<td>0.915</td>
</tr>
<tr>
<td>Spicy Foods</td>
<td>0.909</td>
<td>0.411 – 2.013</td>
<td>0.814</td>
</tr>
<tr>
<td>Acidic Fruits</td>
<td>1.371</td>
<td>0.620 – 3.034</td>
<td>0.435</td>
</tr>
<tr>
<td>Coffee</td>
<td>5.189</td>
<td>1.364 – 19.735</td>
<td>0.009</td>
</tr>
<tr>
<td>Energy Drinks</td>
<td>0.274</td>
<td>0.102 – 0.735</td>
<td>0.008</td>
</tr>
<tr>
<td>Artificial Sweeteners</td>
<td>0.647</td>
<td>0.289 – 1.449</td>
<td>0.288</td>
</tr>
<tr>
<td>Beverage Drinks</td>
<td>0.672</td>
<td>0.304 – 1.486</td>
<td>0.325</td>
</tr>
<tr>
<td>Grains</td>
<td>0.526</td>
<td>0.237 – 1.166</td>
<td>0.112</td>
</tr>
<tr>
<td>Starches</td>
<td>0.511</td>
<td>0.224 – 1.166</td>
<td>0.109</td>
</tr>
<tr>
<td>Psychological Stress</td>
<td>1.656</td>
<td>0.745 – 3.683</td>
<td>0.215</td>
</tr>
<tr>
<td>Sleep Disturbance</td>
<td>2.054</td>
<td>0.896 – 4.708</td>
<td>0.087</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>6.900</td>
<td>2.337 – 20.370</td>
<td>0.001</td>
</tr>
<tr>
<td>Menopausal Women</td>
<td>4.622</td>
<td>1.864 - 11.459</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Antibiofilm inhibition of Hawthorn extracts against UTI causing bacteria

The chemical constituents of Hawthorn are evaluated and represented in table 4. All extracts of Hawthorn indicated the presence of many metabolites such as saponins, terpenoids, flavonoids, alkaloids, polyphenols, tannins, quinines, and alkaldoid. On the other hand, steroids and cardiac glycosides absent in all extract.

Table 4 Phytochemical analysis of Hawthorn fruits and leaves extracts. (-) Negative detection, (+) Positive detection.

<table>
<thead>
<tr>
<th>Active Substances</th>
<th>Aqueous Hawthorn Fruits (AHF)</th>
<th>Aqueous Hawthorn Leaves (AHL)</th>
<th>Ethanol Hawthorn Fruits (EHF)</th>
<th>Ethanol Hawthorn Leaves (EHL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinines</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Phytochemical Screening of Hawthorn ethanolic and aqueous extracts

The effects of hawthorn extracts on the inhibition of biofilm formation are shown in table 5. Percentage inhibition values above 50% showed a high activity. Hawthorn extracts showed a variant activity on the inhibition of attachment. Ethanolic fruits showed a good prevention of biofilm formation of S. aureus, S. epidermidis and E. faecalis. While hawthorn aqueous fruits prevented biofilm formation of S. aureus, E. coli and K. pneumonia. Aqueous leaves showed a good prevention against E. coli and K. pneumonia. However, they showed a low activity against S. aureus, S. epidermidis and E. faecalis, and enhanced biofilm attachment and growth of S. marcescens. Moreover, ethanolic leaves exhibited good prevention against S. aureus, S. epidermidis and E. faecalis and poor activity against K. pneumonia; however, enhanced biofilm formation against E. coli and S. marcescens.
Table 5 Effect of hawthorn extract on various bacterial biofilms.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>S. aureus</th>
<th>P-Value</th>
<th>S. epidermidis</th>
<th>P-Value</th>
<th>E. faecalis</th>
<th>P-Value</th>
<th>K. pneumonia</th>
<th>P-Value</th>
<th>S. marcescens</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous Fruits</td>
<td>59 ± 1</td>
<td>0.000</td>
<td>43 ± 8.8</td>
<td>0.014</td>
<td>30 ± 5</td>
<td>0.009</td>
<td>60 ± 5</td>
<td>0.002</td>
<td>-55 ± 13.2</td>
<td>0.019</td>
</tr>
<tr>
<td>Aqueous leaves</td>
<td>51 ± 3.6</td>
<td>0.005</td>
<td>55 ± 3.6</td>
<td>0.002</td>
<td>40 ± 10</td>
<td>0.02</td>
<td>51 ± 2.6</td>
<td>0.001</td>
<td>69 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Ethanol leaves</td>
<td>67 ± 2.6</td>
<td>0.001</td>
<td>73 ± 2.6</td>
<td>0.000</td>
<td>74 ± 5.2</td>
<td>0.002</td>
<td>-13 ± 2</td>
<td>0.008</td>
<td>28 ± 2.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Ethanol leaves</td>
<td>66 ± 5.2</td>
<td>0.002</td>
<td>-55 ± 13.2</td>
<td>0.019</td>
<td>66 ± 5.2</td>
<td>0.002</td>
<td>-41 ± 8.5</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effects of aqueous extract of hawthorn Fruits (AF) and leaves (AL) and ethanolic extract of hawthorn fruits (EF) and leaves (EL) against bacterial isolates

The diameter inhibition zone values of the hawthorn extracts are shown in table 10. The ethanolic extract of hawthorn fruit and leaves had highest antibacterial activity against Gram positive bacteria and had no effect against Gram negative bacteria. While, for aqueous extract of hawthorn fruit exhibited effective antibacterial activity against S. aureus, E. coli and K. pneumonia; however, the aqueous extract of hawthorn leaves exhibited antibacterial activity against E. coli and K. pneumonia.

Table 6 Antibacterial activity of Hawthorn extracts by the agar well diffusion method, and the values represent means ± SD. “−”: not detected

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Diameter of the Inhibition Zone (mm)</th>
<th>EF</th>
<th>P-value</th>
<th>EL</th>
<th>P-value</th>
<th>AF</th>
<th>P-value</th>
<th>AL</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>13.3 ± 0.57</td>
<td>0.001</td>
<td>12.3 ± 0.57</td>
<td>0.001</td>
<td>11.6 ± 0.57</td>
<td>0.001</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>13.3 ± 1.5</td>
<td>0.004</td>
<td>14.0 ± 1.0</td>
<td>0.002</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. faecalis</td>
<td>14.6 ± 0.57</td>
<td>0.001</td>
<td>14.6 ± 0.57</td>
<td>0.001</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>—</td>
<td>—</td>
<td>12.0 ± 1</td>
<td>0.002</td>
<td>11.3 ± 0.57</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>—</td>
<td>—</td>
<td>11.3 ± 0.57</td>
<td>0.001</td>
<td>11.6 ± 0.57</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. marcescens</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibacterial activity of aqueous and ethanolic extract of Hawthorn

Figure 2 Antimicrobial activity of aqueous and ethanolic extract of Hawthorn extract against UTI causing bacteria (p value=0.001)
Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of hawthorn extracts against UTI causing bacteria:

The MIC and MBC values of extracts were determined in order to compare the antibacterial activities of extracts. The ethanolic extract of Hawthorn fruits and leaves had a minimum MIC 100 mg/ml and MBC 200 mg/ml against S. aureus and S. epidermidis, while it had a minimum MIC 50 mg/ml and MBC 100 mg/ml against E. faecalis and the ratio of MBC/MIC equal to 2. However, the aqueous extract of hawthorn fruits had antibacterial activity against E. coli and K. pneumoniae with MIC and MBC 200 mg/ml. These results similar to aqueous extract of hawthorn leaves had antibacterial activity against E. coli and K. pneumoniae with MIC and MBC 100 mg/ml and the ratio of MBC/MIC equal to 1.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanol Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fruit</td>
<td>Leaves</td>
</tr>
<tr>
<td>S. aureus</td>
<td>MIC 100, MBC 200</td>
<td>R 2, MBC 200</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>MIC 50, MBC 100</td>
<td>R 2, MBC 200</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>MIC 100, MBC 200</td>
<td>R 2, MBC 200</td>
</tr>
<tr>
<td>E. coli</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

DISCUSSION

Urinary tract infection is a very common infection caused by a wide range of bacteria, and characterized by a severe outcome and a higher prevalence in women (12). This infection has the ability to evolve into other body parts including urethra, ureters, bladder and kidneys. According to the world Health Organization (WHO), 60% of risk factors related to the individuals’ health are correlated to their lifestyle, demographic (age, race, gender...) (9). Various ways of lifestyle such as smoking, unhealthy diet, alcohol consumption, and psychological stress (anxiety, stress, depression and sleep disturbance), medical conditions (diabetes, hypertension, hyperlipidemia, heart failure...) are known to correlate with UTI (15). This study assesses the prevalence, factors associated with UTI, and the effect of antibacterial activity of hawthorn against some microorganisms causing UTI.

Our results showed an increased prevalence of UTI in obese patients due to inability of exerting sufficient pressure to empty the bladder. Al-Rubeaan et al. reported an association between obesity and UTI, by stimulating metabolic disorders like diabetes that enhance growth of UTI causative microorganisms (11), against E. faecalis and the ratio of MBC/MIC equal to 2. However, the aqueous extract of hawthorn fruits had antibacterial activity against S. aureus, E. coli and K. pneumoniae with MIC and MBC 200 mg/ml. These results similar to aqueous extract of hawthorn leaves had antibacterial activity against E. coli and K. pneumoniae with MIC and MBC 200 mg/ml and the ratio of MBC/MIC equal to 1.

In the same context, patients with diabetes are highly susceptible to uropathogen invasion by bacteria, due to the fact that glucose is an enhancer of their growth. Moreover, diabetic patients exhibit irritation, immunity alteration, and neutrophil invasion by bacteria, due to the fact that glucose is an enhancer of their growth. In the same context, patients with diabetes are highly susceptible to uropathogen invasion by bacteria, due to the fact that glucose is an enhancer of their growth. In the same context, patients with diabetes are highly susceptible to uropathogen invasion by bacteria, due to the fact that glucose is an enhancer of their growth.
Moreover, our results showed that caffeine intake could increase the risk of UTI infection. This finding is not surprising as caffeine acts as diuretic, thus leading to dehydration, which increase excretion of sodium and lead to overtactive bladder. Consequently, urinary retention can occur and could develop into an infection (1,14).

A study by Maserejian et al. reported that people who drink more than two cups of coffee per day had two-fold significant UTI than those who don’t (14). In a recent study, it was shown that vitamin D deficiency correlates with UTI, because vitamin D is a very potent stimulator to the antibacterial peptide cathelicidin expressed in macrophages and monocyte (8). However, other factors like hypertension, hyperlipidemia thyroid disease, smoking, etc., didn’t show any influence on the infection. This finding is opposing with other studies proving a correlation between UTI and contraceptives. The difference in these findings can be explained by differences in patient selection, study design, number of samples (14).

Hawthorn is a thorny tree belonging to the Rosaceae family with an important medical activity (4). Hawthorn extract exhibited a good effect in promoting digestion, improving immune system and reducing inflammation (10). The chemical constituents identified in hawthorn extract displayed an important defense against bacterial pathogenicity. Previous studies reported that Hawthorn extracts showed an antibacterial activity most particularly against Gram-positive. The high resistance of Gram-negative bacteria is due to the double-layered cell wall composed of outer membrane and lipopolysaccharide (22).

The method of extraction of Hawthorn plant is known to affect the antimicrobial activity (21). Ethanolic extract of hawthorn fruits and leaves exhibited a strong inhibitory effect on E. faecalis, S. aureus, S. epidermidis, while the aqueous extract of hawthorn fruits and leaves has strong inhibitory effect on E. coli and K. pneumonia. The antibacterial activity of ethanolic and aqueous extracts of hawthorn fruits against S. aureus and the aqueous extract of hawthorn fruits against E. coli is in agreement with a study by Zhang et al. Moreover, the results of antibacterial activity of aqueous extract of hawthorn fruits against K. Pneumonia was in agreement with the study done by Niu et al. (22). P. aeruginosa was highly resistant to all plants extracts while E. coli was highly susceptible to both ethanolic extract of hawthorn fruits and leaves. In general, our study showed that ethanolic extract of hawthorn compound exhibited a better antibacterial activity than the aqueous extract. This could be explained by the increase in the extraction rate of phenols, and the interactions of flavonoids especially catechins with hydrophobic compounds in the internal membrane. Consequently, a reduction in the fluidity and the flexibility of the inner and outer cellular membrane can occur, and an inhibition of topoisomerases and helicase enzymes, whereby cell division in blocked (22).

Despite the efficacy of antibiotics against many bacterial infections, the problem of bacterial resistance is still growing, and adverse drug reactions are still challenging. Herbal compounds present a lesser rate of resistance compared with antibiotic therapy (2). Hence, the research and development of antibacterial based on herbal medicines is of great clinical impact. Our study highlighted a promising role of Hawthorn extracts against resistant UTI-causing strains. Future studies are needed to investigate in depth the main components of hawthorn extract involved in this activity.

CONCLUSION

This study shed the light on the risk factors associated with UTI among Lebanese women. The antibacterial activity of hawthorn extracts against many bacterial strains was confirmed, and seems to be affected by the method of extraction. Future studies are needed to evaluate the toxicity of hawthorn extracts, and investigate its prospective therapeutic effect, as a solution for bacterial resistance to antibiotics.

Data Availability: The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Statement: The authors did not receive any funding for this manuscript.

REFERENCES


