USING AQUEOUS EXTRACT OF MALVA SYLVESTRIS AS INHIBITOR FOR THE GROWTH OF SOME MICROORGANISMS THAT CAUSE URINARY TRACT INFECTIONS

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ABSTRACT
Pharmacological properties of Malva sylvestris that had been studied and tested. Our study investigations showed that there are a lot of rich antimicrobial and antioxidants actives compounds of M. sylvestris and they are due to the presence of many unique compounds such as anthocyanin in plant parts precisely in leaves. It was clear that the three different bacterial strains Escherichia coli, Staphylococcus saprophyticus and Pseudomonas aeruginosa were obtained and isolated from infected people were the most available bacteria that cause Urinary tract infections (UTIs). In this study percentage of Escherichia coli was (80%) followed by Staphylococcus saprophyticus (14%) and pseudomonas aerugenosa was (6%) respectively. Three type of aqueous extracts with fact of four treatments (M1, M2, M3 and M4) were done to get synergistic activity, cold water extract for fresh leave plant sample (M1), hot water extract for fresh leave plant sample (M2), hot water extract for dry power of leave plant sample (M3), the mixed extracts M4 was done from the three water extracts (M1+M2+M3) and three concentrations were done for each treatment and they were 25%, 50%, 100%. There was a significant deference among all concentrations of treatments M1, M2, M3 and M4 toward each pathogenic bacterium. Antimicrobial activity was done by using disc diffusion method. Active compounds were detected and phenolic content was determined by spectrophotometer with absorbance values measured at 765 nm. The results showed that the inhibition zones were increased with the increasing of concentrations of Malva sylvestris extract treatment. Results of this study encourage as to use alternative sours for curing like some plants to heal the human illness and to be more responsible towards our self and nature by using natural, cheaper, non-chemical no poising drugs, and give great attention for plants as alternative sours of antimicrobial activity.

KEY WORDS: aqueous extracts, Urinary tract infections (UTIs), Malva sylvestris, synergistic activity.

INTRODUCTION
Urinary tract infections (UTIs) are defined as the presence of microbial pathogens in the urinary tract with associated symptoms. UTIs are one of the most common bacterial infections; Urinary Tract Infections (UTIs) is an infection caused by the presence and growth of microorganism anywhere in the urinary tract and is perhaps the single commonest bacterial infection of mankind[1-2]. When bacteria from the rectal area enter the urinary tract via the urethra to the bladder and multiply in the urine, an infection occurs[3]. Most infections arise from one type of bacteria, E. coli which normally lies in the colon. The organisms most commonly responsible for catheter-associated UTIs are E. coli, Proteus mirabilis, P. aeruginosa, and Streptococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Mycobacterium tuberculosis, Actinomycetes, Nocardia, Candida etc. can cause UTI. In addition Mycoplasma and Chlamydia may be associated with sexually transmitted UTI[4,5]. New research has shown the promising use of medicinal plants: in vitro studies with hydroalcohol extract from many plants for example Myracidron urundeuva, Psidium guajava and Malva sylvestris and they showed potential antimicrobial activity against the microorganisms, antifungal activity on Candida strains[6].

Malva sylvestris belong to the family of Malvaceae and it is species for the genus. Known as common mallow in English speaking[7,8,9,10]. The pharmacological properties of Malva sylvestris are especially due to the presence of anthocyanins in its leaves. Malva sylvestris is justified by the complexity of its composition, which consists of tetrahydroxilated sesquiterpenes and diterpenes, two monoterpenes, six normal-C13 terpenes and eleven aromatic compounds[11]. Similarly to the anthocyanin in the leaves, which has a natural potential for degrading free radicals, it serves as antioxidant, reducing total cholesterol, triglycerides in the blood and preventing thrombosis and cardio-cerebral angiopathy[12].

Malva sylvestris extracts are reported for their radical scavenging effect[13]. Previous chemical investigations have shown that there are a lot of rich antimicrobial and antioxidants presence at the actives compounds of M. sylvestris such as flavonols, ferulic acid, hydroxyxynamic acids, sterols, sesquiterpenes, mono and diterpenes in leaves and stems of[14, 15, 16, 17].
Malva sylvestris as inhibitor for microorganisms that cause UTI

MATERIALS & METHODS
Preparation of plant material
Plants were collected during (February-may) from different parts in Baghdad. Parts of fresh samples were washed and shade dried to obtain 50 gram fresh dried sample. Ground plant materials were used for extraction.

Preparing Bacterial strains
Three different bacterial strains, Escherichia coli, Staphylococcus saprophyticus and Pseudomonas aeruginosa were obtained and isolated from infected people. The isolated bacteria were identified and their characteristic form were done include Gram stain test then the microscopic examination, motility test and biochemical tests were examined according to Cheesbrough\(^\text{[19, 20]}\). The isolates were identified\(^{[21]}\). The strains were maintained on Nutrient agar slants.

WATER EXTRACTION
Three type of extracts were done cold water extract for fresh leave plant sample (M1), hot water extract for fresh leave plant sample (M2), hot water extract for dry power of leave plant sample (M3). also three concentrations were done for each extract and they were 25%, 50%, 100%.

a) Preparing treatment (M1)
Cold extraction was done by adding 500 ml of Distal Water (DW) for 100 gm of fresh leave plant sample then incubate it for 24 hr. in shaker incubator at 35°C then filter the extract by filter paper and then centrifuged the pure liquid for 3000 r/min, after that the supernatant was concentrated by rotary evaporator at 45°C and then the extract was dried by oven at 37°C for 24 hr.

b) Preparing treatment (M2)
Hot extraction was done by adding 500 ml of hot Distal Water (DW) for 100 gm of fresh leaves plant sample then incubate it for 30 min. in shaker incubator at 35°C then the extract was filtered by filter paper and then centrifuged the pure liquid for 3000 r/min, after that the supernatant was concentrated by rotary evaporator at 45°C and then the extract was dried by oven at 37 °C for 24 hr.

c) Preparing treatment (M3)
Hot extraction was done by adding 500 ml of boiling Distal Water (DW) for 100 gm of dried leaves of plant samples then incubate it for 30 min. in shaker incubator at 35 °C then the extract was filtered by filter paper and then centrifuged the pure liquid for 3000 r/min, after that the supernatant was concentrated by rotary evaporator at 45 °C and then the extract was dried by oven at 37 °C for 24 hr.

Preparing the control treatment (C)
The ciprofloxacin antibiotic was considered to be the control at all experiments against all the pathogenic bacteria in the study.

Preparing the mixed treatment M4 (M1+M2+M3)
Best antimicrobial activity against every pathogenic studied bacteria of extract concentration was chosen for each one and then mixed extractions were done from the both Extractions (the two hot extractions and the cold extraction for the plant leaves). The mixed was (M1:M2:M3), (1:1:1), to get a synergistic activity for the mixed treatment.\(^{[22]}\)

Antimicrobial activity measuring
Disc diffusion method was done for this purpose. The Malva sp. extract and ciprofloxacin antibiotic stocks were also made at 25 μg/ml the concentration of the Oflaxacin served as positive controls. Autoclaved discs were loaded with 10 L of the respective plants extract, or ciprofloxacin antibiotic only were prepared then all disks air dried for 5 minutes. The nutrient agar plates were spread with 100 L of respective culture with the help of glass spreader and the loaded discs were placed onto the surface of agar. The plates were left to dry for 5 min and kept in incubator at 37°C for 24 h. The results were seen as zone of inhibition which was measured in millimeters was determined.\(^{[23, 22]}\)

Active compounds Detecting and determination of phenolic content.
Chemical compounds analysis of the Malva sp. extracts were carried out by using the chemical classic methods according to Harborn\(^{[24]}\) total phenolic content was determined according to the method of Singleton\(^{[25, 26]}\). Standard curve was made using Gallic acid as a standard. Different concentrations of Gallic acid were prepared in distilled water, and their absorbance values were measured at 765 nm. Samples measurement as in:

5 ml (phenol reagent) + 900 ml (DW) + 100 ml (extract) then after 5 min we add 4 ml (Na2CO3 15%) incubated 120 min>>> then samples measured at Abs. 765 nm

Statistical Analysis
The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study, all data measuring as triplicate measures\(^{[27]}\).
RESULTS & DISCUSSION
Bacteria isolated caused Urinary tract infections (UTIs)
UTIs are caused by many microorganisms, including gram positive like Staphylococcus and gram negative such as E coli and pseudomonas sp. at this study Escherichia coli was (80%) current and isolate followed by Staphylococcus saprophyticus (14%) and pseudomonas areugenosa (6%) respectively. This finding is similar to many reports which indicated that gram negative bacteria are mostly appeared and also the commonest pathogens isolated from patients with urinary tract infections (Figure 1).

Active compounds Detecting
Aqueous extracts of Malva flowers showed a lower radical scavenging ability compared to the leaves, contrary to being more active against the tested pathogenic microorganisms. Finally, the kind of mallow analysed here can be considered as good sources of some phenolic and antioxidant compounds [28].

Active substance in fruits and vegetables such as phenolic compounds has antioxidant activity as it shown in table (1) the M1 extract containing Flavonoids (fla), Tannins (tan) and Phenols (phe). M2 contane alk, fla, tan, ter and phe. Also we found that M3 had alk and phe. But M4 was the rich extract in studied active compound except terpenes (ter). Total phenolic contents of Malva sylvestris extracts samples are presented in Table (2).

<table>
<thead>
<tr>
<th>Malva sylvestris Extracts</th>
<th>Alkaloids (alk)</th>
<th>Flavonoids (fla)</th>
<th>Tannins (tan)</th>
<th>Terpenes (ter)</th>
<th>Phenols (phe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>_</td>
<td>++</td>
<td>_</td>
<td>_</td>
<td>++</td>
</tr>
<tr>
<td>M2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>M3</td>
<td>+</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>M4</td>
<td>++</td>
<td>++</td>
<td>_</td>
<td>_</td>
<td>++</td>
</tr>
</tbody>
</table>

TABLE 2: Total phenols in the extracts of Malva sylvestris sample concentration (g/ml) determined by using standard curve of Gallic acid

<table>
<thead>
<tr>
<th>The study treatments</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols concentrations (g/ml)</td>
<td>160.14</td>
<td>120.21</td>
<td>83.51</td>
<td>220.30</td>
</tr>
</tbody>
</table>

FIGURE 2: Total phenols in the extracts of Malva sylvestris sample concentration (g/ml) determined by using standard curve of Gallic acid.

Malva sp. extracts antimicrobial activity and measuring inhibition zones
The inhibition zones were determined for each extract in the study and also the inhibition zones were measured for antibiotic activity against UTIs bacteria which considered to be the control treatment. There is a relation between the type of the extract and the active compounds abundance that we can get from the plant parts (Table-1). The
pharmacological properties of *Malva sylvestris* are especially due to the presence of anthocyanins in its leaves. Parts of Malva plant extracts were found to be more active toward pathogenic microorganisms specially gram positive and negative bacteria but no one of the extracts showed an inhibition against Fungi (mold and yeast). Thus, the *M. sylvestris* extracts showed no antifungal activity. These results were comparable to the one reported by Fatima *et al.* (2013) considering the antimicrobial activity of *M. sylvestris* work in widespread on pathogenic bacteria.[29]

**TABLE 3:** The inhibition zones (mm) of cold fresh leave extracts of *M. sylvestris* (M1) Against UTIs microorganisms.

<table>
<thead>
<tr>
<th>(UTIs) bacteria</th>
<th>Inhibition zone (mm) of M1 25%</th>
<th>Inhibition zone (mm) of M1 50%</th>
<th>Inhibition zone (mm) of M1 100%</th>
<th>Inhibition zone (mm) of C (control)</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>5.0</td>
<td>8.0</td>
<td>10.0</td>
<td>15.0</td>
<td>3.071 *</td>
</tr>
<tr>
<td>P. areugenosa</td>
<td>6.5</td>
<td>9.4</td>
<td>11.5</td>
<td>16.0</td>
<td>3.668 *</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>7.2</td>
<td>9.6</td>
<td>12.0</td>
<td>18.0</td>
<td>4.052 *</td>
</tr>
<tr>
<td></td>
<td>* (P&lt;0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4:** The inhibition zones (mm) of hot fresh leave extracts of *M. sylvestris* (M2) Against UTIs microorganisms.

<table>
<thead>
<tr>
<th>(UTIs) bacteria</th>
<th>Inhibition zone (mm) of M2 25%</th>
<th>Inhibition zone (mm) of M2 50%</th>
<th>Inhibition zone (mm) of M2 100%</th>
<th>Inhibition zone (mm) of C (control)</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>3.0</td>
<td>4.0</td>
<td>6.0</td>
<td>15.0</td>
<td>2.983 *</td>
</tr>
<tr>
<td>P. areugenosa</td>
<td>3.4</td>
<td>4.5</td>
<td>5.5</td>
<td>16.0</td>
<td>3.056 *</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>4.0</td>
<td>5.0</td>
<td>7.5</td>
<td>18.0</td>
<td>3.921 *</td>
</tr>
<tr>
<td></td>
<td>* (P&lt;0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5:** The inhibition zones (mm) of hot dry leave extracts of *M. sylvestris* (M3) Against UTIs microorganisms.

<table>
<thead>
<tr>
<th>(UTIs) bacteria</th>
<th>Inhibition zone (mm) of M3 25%</th>
<th>Inhibition zone (mm) of M3 50%</th>
<th>Inhibition zone (mm) of M3 100%</th>
<th>Inhibition zone (mm) of C (control)</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>3.0</td>
<td>4.8</td>
<td>7.0</td>
<td>15.0</td>
<td>3.169 *</td>
</tr>
<tr>
<td>P. areugenosa</td>
<td>4.0</td>
<td>6.0</td>
<td>8.0</td>
<td>16.0</td>
<td>3.553 *</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>6.0</td>
<td>8.0</td>
<td>9.5</td>
<td>18.0</td>
<td>3.703 *</td>
</tr>
<tr>
<td></td>
<td>* (P&lt;0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In table (1) there was a significant deference among all concentrations of M1 treatment toward each pathogenic bacteria, in the treatment M1 the results showed that the inhibition zones were increased with the increasing of concentrations of the M1 extract treatment. The best concentration was at 100% against *E. coli, P. areugenosa, S. saprophyticus* with inhibition zones of (10, 11.5, 12) mm respectively (table 3). There was a significant deference noticed among all concentrations at these two treatment. Inhibition zones of the treatment M2 and M3 were also increased with the increasing of extract concentration (Table 4, 5), but it appeared that the studied bacteria were less sensitive to these two treatments because the amount of active compound especially total phenols which considered to be the most important compound that is responsible for the activity of the plant extracts table (2).

**TABLE 6:** The inhibition zones (mm) of mixed extracts of *M. sylvestris* (M4) Against UTIs microorganisms.

<table>
<thead>
<tr>
<th>(UTIs) bacteria</th>
<th>Inhibition zone (mm) of M4 25%</th>
<th>Inhibition zone (mm) of M4 50%</th>
<th>Inhibition zone (mm) of M4 100%</th>
<th>Inhibition zone (mm) of C (control)</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>6.2</td>
<td>8.6</td>
<td>11.6</td>
<td>15.0</td>
<td>3.416 *</td>
</tr>
<tr>
<td>P. areugenosa</td>
<td>6.0</td>
<td>10.0</td>
<td>12.5</td>
<td>16.0</td>
<td>2.963 *</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>9.6</td>
<td>11.4</td>
<td>15.5</td>
<td>18.0</td>
<td>3.375 *</td>
</tr>
</tbody>
</table>

The result of treatment M4 gave the maximum inhibiting diameter towards the pathogenic studied bacteria which were sensitive to this treatment because of the interaction and synergetic activity among these three kinds of extracts also the phenols compound was 220.30 g/ml this result was the highest among all extracts and the highest inhibitions zones were for the 100% concentration (11.5, 12.5 and 15.5) mm against *E. coli, P. areugenosa and S. saprophyticus* respectively (Table 6). The results of this study was similar to the results of a study by (aljanabi *et al.*, 2011) who connect the activity of plant antimicrobial with present of phenolic acids and they are play a very important role of causing damage in DNA preventing diseases[30,31]. This study was agree with study by[32,33] and those who showed the importance of the leave aqueous extract as a treatment for many states. There are a lot of plant used as antimicrobial such as a study by[34] which was agree with the aim with present study by giving the importance and a site of the using of some selected plants that have been widely interred in the management of
various human illnesses in the past and present time. A study by Meena et al. (2014) [35] share the opinion with this study and they revealed that phytochemical analysis shows the presence of alkaloids, tannins, flavonoids and saponins in various extracts derived from leaves, stem and seeds of plant these active components were behind the anti-sickling and antimicrobial activity possessed by leaves [36]. A result of the study was in corresponding with a study by Sabri et al., 2012 [37]. Revealed that extracts of Malva sylvestris L. contain alkaloids, flavonoids, tannins, starch, saponins, sterols and steroids and anthocyanosids which give the Malva healing properties. Malva sylvestris is a good source for natural foods supplements, pharmaceutical industry purposes and for organic food rich with antioxidant compounds [38]. All these studies and experiments encourage as to use alternative sours of cure like some plants to heal the human illness and to be more responsible towards our self and nature by using natural, cheaper, non-chemical non poising drugs, and give great attention for plants as alternative sours of antimicrobial activity.

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