Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN: 2521-3520 (online)
Volume 28, Issue 3, 2017
DOI: http://doi.org/10.23851/mjs.v28i3.177

Research Article

The Antimicrobial Activity of Some Medical Plants Extracts Used Against Some Types of Bacteria that Causes Urinary Tract Infection

Nehia Hussein, Noor A. Hanon
Biotechnology Department, College of Applied Science, University of Technology, IRAQ
*Correspondent Author Email: nehiahussein@yahoo.com

ArticleInfo

Received
3 Apr. 2017

Accepted
17 Oct. 2017

Abstract

This study was done to evaluated the antibacterial activity of hot ethanolic and aqueous extracts of *Syzygium aromatic* (*S.aromatic*) and *Quercus infectoria infectoria* (gall) against pathogenic bacteria that causing urinary tract infection (UTI). Such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Qualitative detection of active compounds was done with chemical reagents. The result showed that the extracts of *Q.infectoria infectoria* was composed of the saponin, tannins, flavonoids, terpenes, Resins, coumarin, glycosides, alkaloids and volatile oils. Also the Qualitative detection of the active groups was further verified with Fourier Transform Infrared Spectroscopy (FTIR). The sensitivity of bacteria was examined against 12 different antibiotics. The results show that *E.coli* was the most resistant bacteria, resisting 8 antibiotics; *P.aeruginosa* resisted 5 antibiotics; while *S.aureus* resistance to 4 only. The antibacterial activity of the plant extracts was investigated for each extracts against bacteria mention above, the plant extracts showed different effects on the growth of all bacterial strains. The Minimum Inhibitory Concentration (MIC) and the Minimum bactericidal concentration (MBC) of the extracts were determined. The kill- time was determined for each extract.The antioxidant activity of the plants in the study was investigated. Compiled results show that all plant extracts have antioxidant effect but that varied depending on the type of the plant and extract. The toxicity of the plant extracts was examined on human red blood cells. It was concluded that the plants do not exhibit any toxicity except for the extracts of *Q.infectoria* which caused the agglomeration and precipitation of the red blood cell.

Keywords: Medicinal plant, Antimicrobial, Antioxidant, kill time, cytotoxic activity.

الخلاصة

أجريت هذه الدراسة لتقييم التأثير المثبط للمستخلص الايثانولي الساخن والمائي الساخن لنبات *Syzygium aromatic* (*S.aromatic*) و *Quercus infectoria* ضد البكتيريا المسببة للأمراض التي تسبب التهاب المسالك البولية (UTI). مثل *Escherichia coli* و *Staphylococcus aureus* و *Pseudomonas aeruginosa*. تم الكشف عن المركبات الفعالة في النبات باستخدام الفحوصات الكيميائية وقت اظهرت النتائج وجود كل من saponin, tannins, flavonoids, terpenes, Resins, coumarin, glycosides, alkaloids وvolatile oils . كما تم أستخدام جهاز الـ (FTIR) للكشف عن المجاميع الفعالة في المستخلصات. تم فحص حساسية البكتيريا ضد 12 مضادات حيوية مختلفة, أظهرت النتائج أن *E.coli* هي البكتيريا الأكثر مقاومة، حيث اظهرت مقاومة لـ 8 مضادات حيوية من اصل 12 مضاد, اما *P.aeruginosa* فاظهرت مقاومة لـ5 مضادات, بينما اظهرت *S.aureus* مقاومة إلى 4 فقط. تم فحص حساسية البكتريا المذكورة اعلاه للمستخلصات النباتية, أظهرت المستخلصات النباتية تأثيرات مختلفة على نمو جميع سلالات بكتيرية. تم تحديد التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى (MBC) للمستخلصات. تم تحديد وقت القتل time-kill لكل مستخلص. تم التحقيق في النشاط المضاد للأكسدة للنباتات في الدراسة واظهر النتائج أن جميع المستخلصات النباتية لها تأثير مضاد للأكسدة ولكن ذلك يختلف باختلاف نوع النبات ونوع المذيب المستخدم. تم فحص سمية المستخلصات النباتية على خلايا الدم الحمراء البشرية,اظهرت النتائج ان النباتات لا تظهر أي سمية باستثناء مستخلصات *Q.infectoria* التي تسببت في تكتل وترسب كريات الدم الحمراء.

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

Introduction

Urinary tract infection is very common infections in the community (community acquired) and hospital (nosocomial infection) affects different ages, occur as a result of the multiplication of bacteria in the urinary tract [1]. Women are more vulnerable to infection than men because of anatomical variation of urinary tract infections between women and men [2], this is not a common disease among men but it is dangerous if the man was infected. The cystitis is more frequent in pregnant women because of hormonal changes and of increasing size and weight of the uterus during pregnancy and a high rate of progesterone hormone which squeeze on the urinary tract and reduces urinary flow to the bladder. Cystitis reasons vary between one person and another depending on several reasons including age, health condition, immune status, sex and physiological condition [3]. That most common pathogenic that cause UTI is *E.coli* bacteria belonging to Enterobacteriaceae family which cause between 80%-85% non-complex cystitis [4] [5], whereas other bacterial species like a bacterium *Enterobacter cloacae*; *Proteus mirabilis; Klebsiella pneumoniae* cause about 10% while *Staphylococcus saprophyticus* bacteria and Staphylococcus aureus cause 10-15% [6]. Because of the long-term use of antibiotics or use it in wrong way the bacteria became more resistant to the antibiotics [7], to find alternative medicine medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs, or use to complement or neutralize drag's negative effects (synergic medicine), also medical plants can prevent the appearance of some diseases, this will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies [8]. Plants also use as antioxidant which is use treating or getting rid of free radicals which are the first line of defense against free radicals, free radicals are atoms missing electron, causing instability and thus the atom begins to interact with cell components to get stability, and that stability leads to form another free radical and therefore a series of free radicals began [9].

The study aims to detect of the biological effectiveness for the plants extract on the growth of pathogenic bacteria that cause inflammation UTI, Antioxidant activity, cytotoxisity in RBCs, and time-kill for each extract.

Material and Methodology

Collection of bacterial isolates

Bacterial isolates were collected from Educational laboratories in the City of Medicine, Al-Kindi Hospital Laboratories, Al-Alwiya Hospital Laboratories and Al-Alawi Children's Hospital Laboratories) in Baghdad,Iraq. 30 isolates were collected; 10 isolates of each bacteria that used in the study; that diagnosis and isolates with VITEK, and API strips for UTI patients.

Plant collection

Syzygium aromaticum carnivores and *Querus infectoria* were collected from the local market in Baghdad. During October, the plants were classified by Dr. Zeinab Abd-Aoun Department of biology/ College of Science for Girls / University of Baghdad. The plant parts (leaves, fruit) are grinded with the electric grinder.

Preparation of the plant extract

The alcoholic extract was prepared by Soxhlet extraction method. About 100 g of plant powder was packed into a thimble and run in Soxhlet extractor. It was extracted with 70% ethanol for the period of about 7 hr. or till the solvent in the tube of an extractor become colorless. After that extracts were evaporated from extract in rotary evaporator to get the syrupy consistency. Then extract was kept in refrigerator at 4 °C until use [10].

The aqueous extract was prepared by dissolve 50 g of plant powder in 500 ml distil water and

put it in shaking incubator in 70°C for 30 min After that extracts were evaporated from extract in rotary evaporator to get the syrupy consistency. Then extract was kept in refrigerator at 4 °C until use [11].

Qualitative detection of antimicrobial activity of plant extract against bacteria:

Plants extracts were tested for antimicrobial activity via agar well diffusion method against different pathogenic microorganisms, *E. coli*, *P. aeruginosa*, (Gram-negative), *S. aureus* (Gram-positive). Pure bacterial cultures were subcultured on mueller hinton agar (MHA). Each strain was uniformly swabbed onto the individual plates by using sterile cotton swabs. Wells with a diameter of 8 mm were made on nutrient agar plates by using the gel puncture method. A micropipette was used to pour 100µL of each extract solution onto each well on all plates. After incubation at 37°C for 24 h, the zone inhibition diameter was measured in millimeters.

PH measurement of plant extracts:

10 g of plant extract (water and alcohol) solve in 90 ml distilled water and left in the blender for 10 minutes, then measured the pH of the extract using PH meter [12].

Specific chemical detection of active compounds of plant extracts:

Based on routine methods and using reagents for this type of testing.

Fourier transforms infrared spectroscopy:

Samples (vegetable powder) were examined by FTIR device. The sample spectroscopy was performed within the range 400-4000 cm^{-1}, and the results were compared with the tables in.

Antibiotic sensitivity test:

The Kirby Bauer disc diffusion method was used to determine the sensitivity of isolates to antibiotics [13]. Mueller Hinton agar was used in this test, bacterial suspension were prepared and compared with the McFarland standard tube (1.5 x 10^8 cells / ml).

Semi Qualitative detection of antimicrobial activity of plant extracts against bacteria:-

Agar well diffusion was used to determine the effectiveness of plant extracts against isolated bacteria after the preparation of the experimental concentrations (40, 60, 80, 100 mg / ml). The bacterial suspension was presented for each isolation and 0.1 mL of it was spread over medium and the middle hole using the perforated hole. Add 100 µl of each hole with the prepared concentrations of the plant extract, as well as the control hole to which 10% DMSO was added for the alcoholic extract and the distilled water of the aquatic extracts. After incubation at 37 ° C for 24 hours, the inhibitory zone was measured around each well in millimeters, and was recorded as mean ± SD of the triplicate experiment [14].

The antibacterial effect of mixing plant extracts:

Binary mixture was prepared by mixing an equal amount of each plant's extract, then the antibacterial effects test done as mentioned above in qualitative detection of antimicrobial activity of plant extract against bacteria.

The antibacterial effect of mixing extracts with antibiotics in the growth of microorganisms:

the mixture of antibiotics and plant's extract was prepared by immersion the antibiotics discs in plant's extracts, let it dry for 10 min, then the discs placed in a cultured Mueller hinton agar, the plates incubate in 37°C for 24 hr.

Measurement of MIC and MBC for plant extracts:

To calculate the minimal inhibitory concentration (MIC) and the minimum bacterial concentration (MBC) of the plant extracts based on what is stated in and as follows: 0.8 ml of the brain heart infusion broth was added to glass test tubes, In addition, 0.1 milliliters of plant extracts were added with the exception of the control tube. The same size of

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

the normal saline solution was added, and 0.1 mL of the comparative bacterial strain was added with McFarland tube (0.5). After incubation in $37c^0$ in 24 hours, the results were recorded on the basis of turbidity observation. And then took 100 microliters of the mixture and spread on the center of Muller Hanton agar and incubated for 24 hours at a temperature of 37^0C. The results were recorded on the basis of growth (+) or lack of growth (-), the experiments were carried out in triplicates.

Time-Kill for plant extracts:

10 ml of nutrient broth was prepared, then 0.1 ml of plant's extracts and 0.1 ml of bacteria were added to the broth, incubated in shaking incubator (150 rpm) at 37^0 C, estimate bacterial cells concentration at (0, 2, 4, 8, 24) hr [15].

Antioxidant Assay:

The method was adopted in Gentaur, cat. No.ECAT-100, Belgium, with the preparation of a sample of plant extracts with 50 µg / mL concentrations, then transfer 10 µg of sample, 10 µg of positive control and 10 µg of negative control And then add 90 microliters of H_2O_2 with a 50 micromolar concentration to the catalase reactions, incubate for 30 minutes at room temperature and add 100 microliters of reagent. After 30 min of incubation at room temperature, the absorbance was obtained against a blank.

Sample at 490 nm wavelength indicating the color change in the drill effectiveness of the enzyme catalase the degree of color change is strongly related to the effectiveness of the enzyme. All experiments were carried out in triplicates.

Cytotoxicity assay:

The effect of extracts on blood was studied in the blood-agar medium as reported in [16]. The agar-well diffusion method was adopted. Normal saline was used as a control, 0.1 ml of extracted plant and control were added to the plate. After incubation at 37 ° C for 18-24 hours the effect of the extract was determined by measuring the zone of inhibition around each well.

Statistical Analysis:

Analysis of variance (ANOVA) was analyzed in two directions using the SPSS.

Result and discussion

Chemical detection of active compounds in plant extracts:

Table 1 shows the routine detection of the active groups found in the plant extracts. The results were agreed with [17] and [18] where they confirmed that *S.aromatic* is rich in phenolic compounds which cause the biological activity of *S.aromatic*. In Q.infectpria the result show that the *Q.infectoria* is the main component of it by up to 50-70%, this result were agreed with [10] and [20]. The pH value of aqueous and alcohol extract of *S.aromatic* was 6 and 5. 8 respectively. The pH value of aqueous and alcohol extract of the *Q.infectoria* was 7.1 and 6.8 respectively.

Table 1: Chemical components of aqueous and alcohol extracts of *S.aromatic* and *Q.infectoria.*

Active compound	*Syzygium aromatic*		*Q.infectoria infectoria*	
	aqueous	alcoholic	aqueous	alcoholic
saponin	-	-	+	+
flavonoid	+	+	+	+
Terpenes	-	-	+	-
steroids	-	-	-	-
coumarin	+	+	+	+
violet oil	-	+	-	-
tannin	+	+	+	+
resins	+	+	+	+
alkaloids	+	+	+	+
glycoside	+	+	-	-
Phenol	+	+	+	-
pH	6	5.8	7.1	6.8

(+)= present in plant, (-) = not present in plant

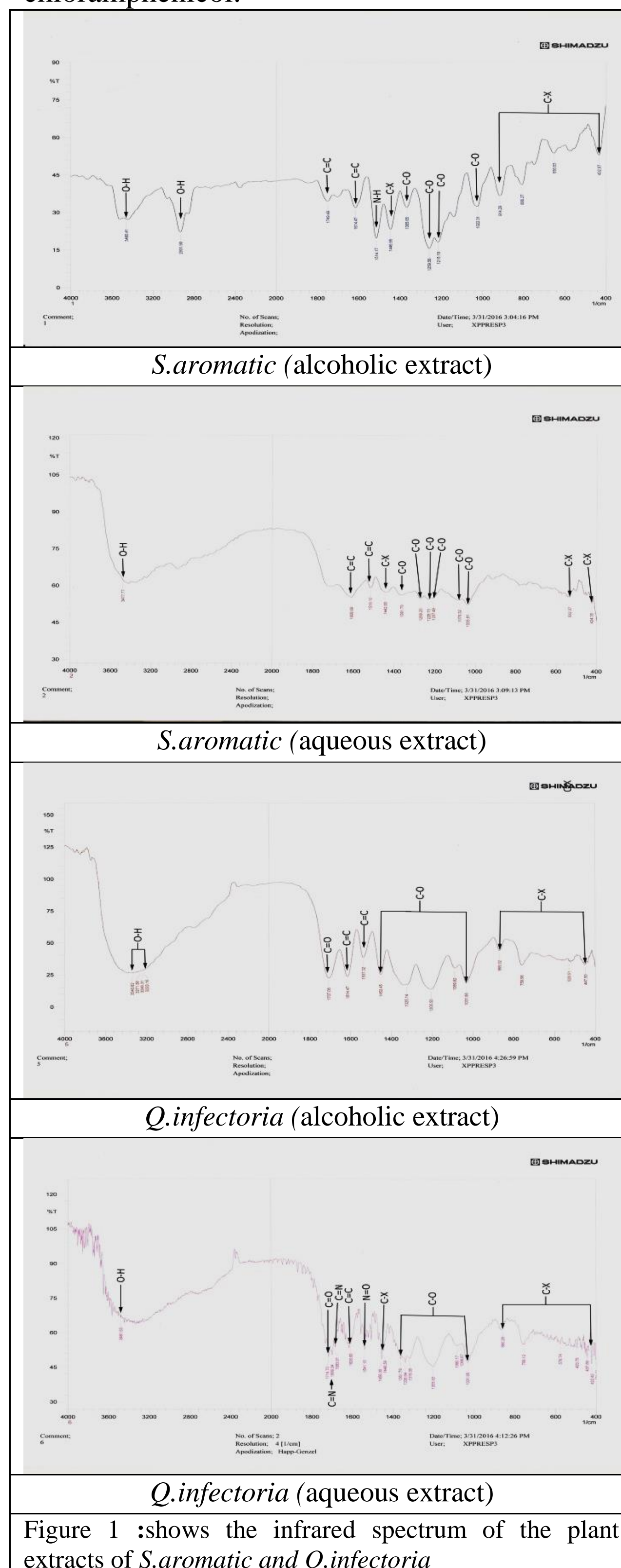
Fourier transforms infrared spectroscopy (FTIR):

The FTIR is a microbiological test that is used to identify chemical elements. Chemical compounds are identified by how the chemical

bonds between the compounds are absorb the infrared; each compound has its own absorptive composition; the spectral absorption results of the extracts show a band at the rang (3313-3931 cm^{-1}) for the alcoholic extract and the range (3317-3481 cm^{-1}) for the aqueous extract which indicates the presence of OH, and the range (1613-1749 cm^{-1}) for the alcoholic and the range (1516-1618 cm^{-1}) for the aqueous extract indicated the presence of double bonds in the form of alkene (C = C), the beams at (1440cm^{-1} - 1446cm^{-1}) for the alcoholic extract and the beam at 1442 cm^{-1} in aqueous extract indicate the presence of fluoride (C-X) in *S.aromatic* , the range (1031-1452 cm^{-1}) indicate the presence of alcohol in alcoholic extracts, and the range (756-1089 cm^{-1}) indicate the presence of alcohol. the beams at the range (644-833 cm^{-1}) of the aqueous extract indicate the presence of chloride (C-X), as indicated by the beams (432-578 cm^{-1}) indicates the present of bromide in the alcoholic extracts of *S.aromatic* and *Q.infectoria*, in aqueous extract, the beam at (424-578 cm^{-1}) indicate the present of bromide in all extracts. Whereas the beam at 1446 cm -1 indicated the present of fluoride in the alcoholic extract of *S.aromatic*, at the rang (2144-1456 cm cm^{-1}) indicated the present of fluoride in aqueous extract of both *S.aromatic* and *Q.infectoria*. While the beams at 1716 cm^{-1} and 1699 cm^{-1} indicate the presence of double beds (C = O), (C = N), respectively, the beams at 1541 cm -1 indicate the presence of nitrates (N = O), as show in the Figure 1.

Sensitivity of bacterial isolates to antibiotics:

The antibiotic susceptibility examination of bacterial isolates was compared to the standard tables mentioned in. The results showed that *E.coli* resistance to Erythromycin, Ciprofloxacin, Cefotaxime, Tobramycin, Doxycycline, and Clindamycin are 100%, 97%, 94%, 100%, 89% and 93% respectively, while both chloramphenicol and gentamicin were inhibited 22.33 mm and 18.66 mm respectively, these results were with [21]. *S.aureus* was less resistant than other bacteria, Carbencillin, Ampicillin and Cefotaxime were 100% resistant and 93% resistant to chloramphenicol.



*S.aromatic (*alcoholic extract)

*S.aromatic (*aqueous extract)

*Q.infectoria (*alcoholic extract)

*Q.infectoria (*aqueous extract)

Figure 1 **:**shows the infrared spectrum of the plant extracts of *S.aromatic and Q.infectoria*

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

While the rate sensitivity to Clindamycin was 29.11 mm, the rate sensitivity to Ciprofloxacin was 25 mm, the rate inhibition zone of Erythromycin and Azithromycin was 20 mm, and these results are in line with previous research done by [22]. *P.aeruginosa* showed high resistance to both Carbencillin, Ampicillin, Clindamycin 100%, Erythromycin 92% and Amikacin 85%, and show highest sensitivity to Ciprofloxacin (45.66 mm) followed by Gentamicin and Azithromycin (both cause 25.55 mm inhibition zone), Tobramycin and Doxycycline both cause 20 mm inhibition zone. This results are in line with previous research done by [23] except Amikacin which cause 19mm inhibition zone on *P.aeruginosa*. The results showed that *E. coli* was the most resistant to antibiotics. It was resistant to 8 of the 12 Antibiotic, followed by *P.aeruginosa*, which was resistant to 5 of the 12 *antagonists*, while *S.aureus* was resistant to 4 Antibiotics of total 12 antagonists (Figure 2).

The effect of plants extract on bacteria:

The test was done by Agar well Diffusion technique, the stock concentration has been dependent, and the antibacterial effect of plants extract was different according to the type of plant extracts. The results showed that all plants which used in the study have good activity on all strain of bacteria at different concentration; as show in Figure 3; the extracts of *S.aromatic* fruits have High inhibitory effect, the alcohol extract was more effective than aqueous extract on the bacterial isolates. The result of antibacterial susceptibility testing showed that all the strains of the three pathogens *S.aureus*, *E.coli* and *P.aeruginosa* were highly susceptible to *S.aromatic* alcoholic extract with average diameter zone of inhibitions of 27.66 mm, 23mm, 19mm respectively, in aqueous extract the average diameter zone of inhibitions was 31mm, 8mm, 14.33 to the *S.aureus*, *E.coli* and *P.aeruginosa* respectively. These results are also evidenced through the work done by [24]. The antibacterial effect in *S.aromatic* extracts is due to the presence of alkaloids and *Q.infectoria* which has an antiseptic effect such as vanillin and Eugenol [25].

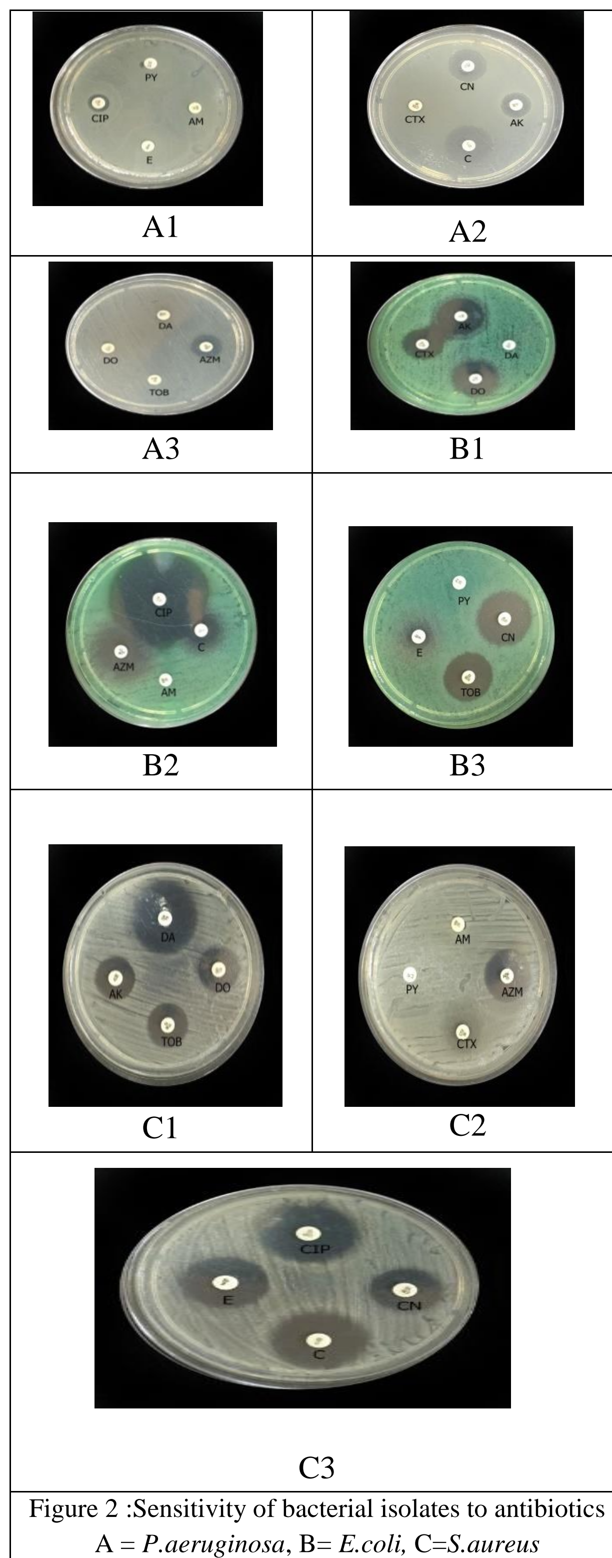


Figure 2 :Sensitivity of bacterial isolates to antibiotics
A = *P.aeruginosa*, B= *E.coli,* C=*S.aureus*

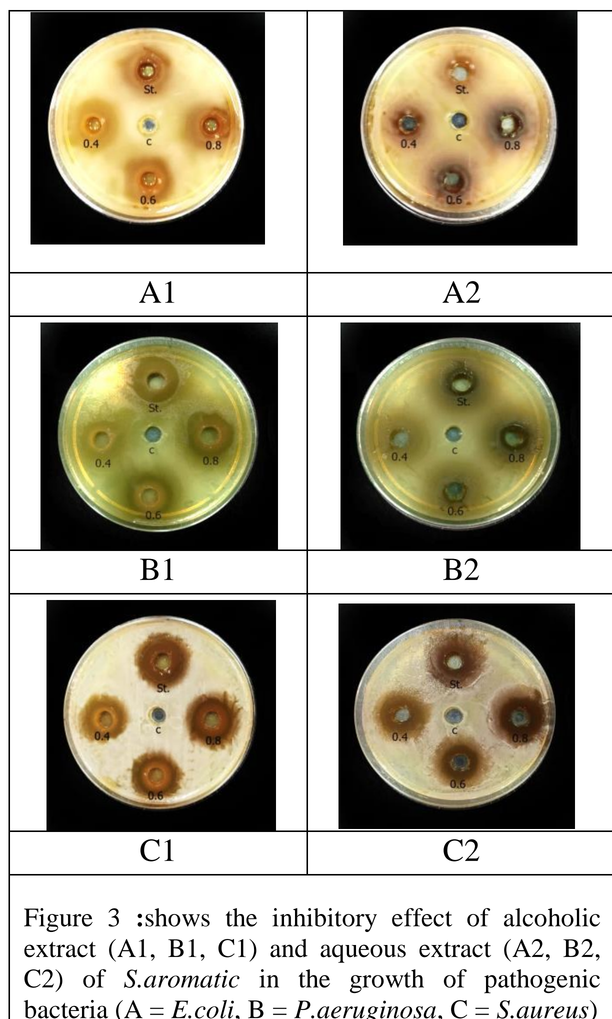


Figure 3 :shows the inhibitory effect of alcoholic extract (A1, B1, C1) and aqueous extract (A2, B2, C2) of *S.aromatic* in the growth of pathogenic bacteria (A = *E.coli*, B = *P.aeruginosa*, C = *S.aureus*)

The results of *Q.infectoria* extracts showed that the extract of alcohol has a higher inhibitory effect than the aqueous extract on *E.coli* and *S.aureus,* and equal in inhibitory on *P.aeruginosa*. Statistical analysis showed significant differences in inhibiting bacterial isolates at P <0.01 at the highest concentration (100 mg / ml), the results showed that the alcohol extract gave the highest effect in the growth of *P.aeruginosa* bacteria with an inhibition diameter of 35 mm, followed by *S.aureus* bacteria with an inhibition rate of 29.66 mm, *E.coli* with an inhibition diameter of 24.66 mm. The results of the treatment with the aqueous extract at the highest concentration showed a significant effect on the inhibition of all isolates at the probability level p <0.01, where the highest inhibition of *P.aeruginosa* was 35mm inhibition diameter followed by *S.auerus* with an inhibition rate of 28.33 mm, And finally in the growth of *E.coli* bacteria at the rate of inhibition region was 11.66 mm, These results are also evidenced through the work done by [26] and [27] (Figure 4).

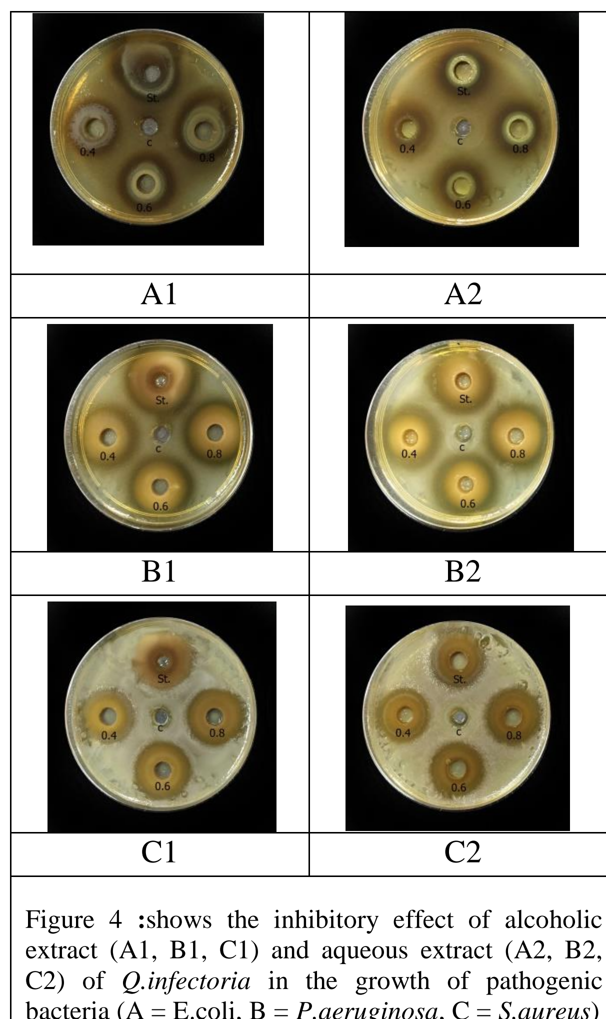


Figure 4 :shows the inhibitory effect of alcoholic extract (A1, B1, C1) and aqueous extract (A2, B2, C2) of *Q.infectoria* in the growth of pathogenic bacteria (A = E.coli, B = *P.aeruginosa*, C = *S.aureus*)

The results indicate that there is a variation in the inhibitory effectiveness of plant extracts towards bacterial isolates. This may be due to variation in extraction method, solvent type and nature of the microbiological membranes [28]. It is worth mentioning that the hot alcohol extract was more effective in compared with aqueous extract, which may be due to the fact that the solubility of active substances in the solvent of alcohol is more than aqueous solvent [29]. When comparing the plant extracts, it was observed that the extract of the alcohol of the

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

Q.infectoria gave the highest inhibitory effect against the bacterial isolates under study. The inhibitory effect of the *Q.infectoria* can be explained by the fact that it contains some active compounds such as alkaloids, turpens, tannins, flavons, resins, Table 1. The saponin work to reduce sugar within the bacteria, where they have a correlation with monocrystalline sugars, leading to cell death [30]. Alkaloids have the ability to penetrate the bacterial cell and interfere with DNA, while the work of tannins inhibit the enzymes and transport proteins found in the cell membrane [31]. Flavonoids also have toxic properties to bacteria, fungi and viruses by forming complexes with proteins [32]. Phenols are compounds that have the ability to form complexes that damage the bacterial cell wall and thus inhibit one or more of the metabolic reactions controlled by those enzymes that may be necessary for the growth and proliferation of bacterial cells or responsible for building different proteins [33]. The difference in effect on bacteria by plant extracts is due to the difference in the chemical composition of the bacterial cell, The Gram positive bacteria contains 90% of peptidoglycan, allowing hydrophobic compounds to easily enter the cell. Gram is more complex, containing less peptidoglycan and an outer wall that surrounds the cell wall and binds to it, thereby increasing the resistance of gram-negative bacteria and determining the permeability of the material into the bacteria [34].

The antibacterial effect of mixing plant extracts:

The results show that the alcoholic mixture are more effective than single extract on *E.coli* and *p.aeruginosa*, but the effect on *S.aureus* show that the single effect of both plant's extracts are more effective than binary mixing extracts, as shown in Figure 5. The aqueous mixture of plant's extracts show less antibacterial effect on *S.aureus* and *p.aeruginosa*, but on *E.coli* the binary effects was higher, as show in Figure 6.

Those increase in effect because the active compounds present in each extract may increase the action of flavons or Benzene or volatile oils due to the increased concentration of compounds. On other hand the decrease in the effect of these mixtures is due to the chemical reaction of the active compounds in each extract which lead to decrease the effective of compounds. Such a case is known in pharmacology when the combination of compounds with each other increases the effectiveness of some while others decrease their effectiveness after mixing.

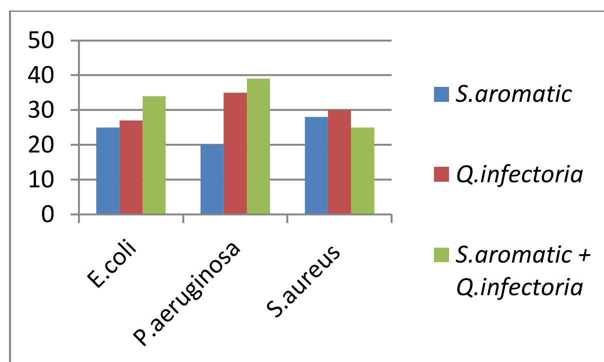


Figure 5: The effect of the mixing of alcoholic plant extracts (mm) and its effect on microorganisms used in the study.

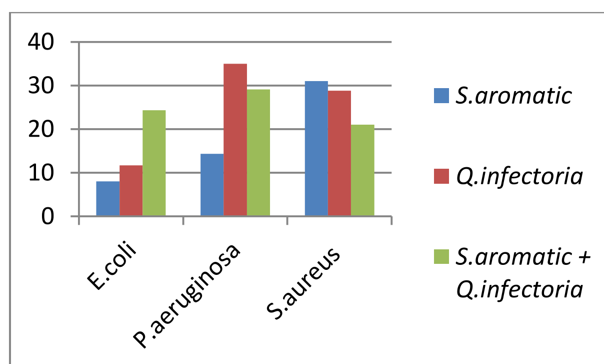


Figure 6: The effect of the mixing of aqueous plant extracts (mm) and its effect on microorganisms used in the study.

The antibacterial effect of mixing extracts with antibiotics in the growth of microorganisms:

Through the process of mixing plant extracts and antibiotics together, no synergistic action was observed, in fact in some cases there where an antagonism effect or no effect on the growth of the microorganisms use in the study, this is clearly shown in following antibiotics CTX, TOB, DO, PY, AM, E, DA.

Determination of the minimum inhibitory concentration and minimum bactericidal concentration of plant extracts:

Minimum inhibitory concentration (MIC) is the lowest concentration inhibits bacteria growth as much as possible. The results showed that the alcohol extract of *S.aromatic* had the strongest effect on the isolates used in the study in compared with quercus. The concentration of 20 mg / mL was the lowest inhibitory concentration of all bacteria used in the study.

The alcoholic extracts of *Q.infectoria* showed a strong effect against *E.coli* and *P.aeurginosa* at a concentration of 20 mg / mL, while the effect was weak against *S.aureus* at 60 mg / mL. In aqueous extracts of *S.aromatic* was weaker than the alcohol extract in affecting *E.coli* at a concentration of 60 mg / mL while the aqueous extract of *S.aromatic* was equal in effect to the rest of the species at a concentration of 20 mg / mL for both extracts. The water extract of the *Q.infectoria* was weaker than the alcohol extract in the *E.coli* effect at 60 mg/ml, while the strongest effect on *S.aureus* bacteria was at 20 mg/ml, while the effect of aqueous extract was equal to alcohol extract of *Q.infecoria* on both inhibite *P.aeruginosa* growth at 20 mg/mL. Minimal bactericidal concentration (MBC) was also determined, depending on the growth or non-growth of bacteria on the agar. The results showed that the MBC was higher than the MIC of plant extracts. The MBC of the *S.aromatic* extract was the strongest effect compared with *Q.infectoria* at concentration of 40 mg / mL for all bacterial species, whereas the alcohol extract of the *Q.infectoria* showed a variation depending on the type of bacteria, The MBC was (40 mg / ml) *E.coli* and *P.aeruginosa*, in S.aureus the MBC was (80 mg/mL). In the aqueous extract, the MBC of *S.aromatic* was the weakest in *E.coli* which was (80 mg / mL). The MBC of both the alcoholic and aqueous extract of *S.aromatic* was equal in effect on both *S.aureus* and *P.aeruginosa* at the concentration (40 mg / mL) for both extracts; the MBC of the *Q.infectoria* was the lowest concentration against E. coli (80 mg / mL) and *S.aureus* and *P.aeruginosa* (40 mg / ml). The anti-extract activity is increased by increasing concentration and the value of MIC and MBC varies depending on the type of extract and bacteria, this test gave the plant compounds the opportunity of movement and action within the medium to influence as many bacteria as possible. The value of the MIC is influenced by the nature of the cell wall, the production of bacteria for certain enzymes and the concentration of bacterial suspension [35].

Time-Kill for plant extracts:

The time of killing for plant extracts was studied to estimate the bactericidal effect of the extract on the studied bacteria. The results showed that aqueous and alcoholic *S.aromatic* extracts showed an equal effect against all bacterial isolates used in the study. The time-kill for *P.aeruginosa*, *S.aureus* and *E.coli* was two hours. For the *Q.infectoria*, the aqueous extract was significantly more effective than the alcoholic extract, which was two hours for the aqueous extract of *P.aeruginosa*, *S.aureus*, *E.coli*, and 4 hours for the alcoholic extract for *P.aeruginosa*, *S.aureus, E.col;* Figure (7 and 8); time-kill of an extract depended on the type of bacteria (growth rate under conditions of exponential growth of bacteria) and the concentration and set of action of ingredients in the extract [36]. It can be said that this is the first study in Iraq. This test is based on the determination of the bactericidal effect of the plant extracts under study, so we could not obtain similar local sources so that the results could be discussed more appropriately.

Evaluation of antioxidant efficacy of plant extracts:

The results showed that *S.aromatic* extract contains a quantity of catalase enzyme. The *S.aromatic* extract contains 210U / L while the aqueous extract contains 165U / L, and these results are in line with [37]. *S.aromatic* extracts can be considered a good source of antioxidants, the antioxidant effect of *S.aromatic* are due to eugenol and eugenyl acetate compounds that are high in *S.aromatic*

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

[38]. The results showed that the *Q.infectoria* contain more amount of catalase compared with *S.aromatic* , and the extracts of *Q.infectoria* contain different proportions of the catalase enzyme according to the type of solvent, the alcoholic extract contains a high percentage of catalase compared to the aqueous extract, alcoholic extract contain 410 U/L while the aqueous extract contains 95 U/L, as shown in Table 2.

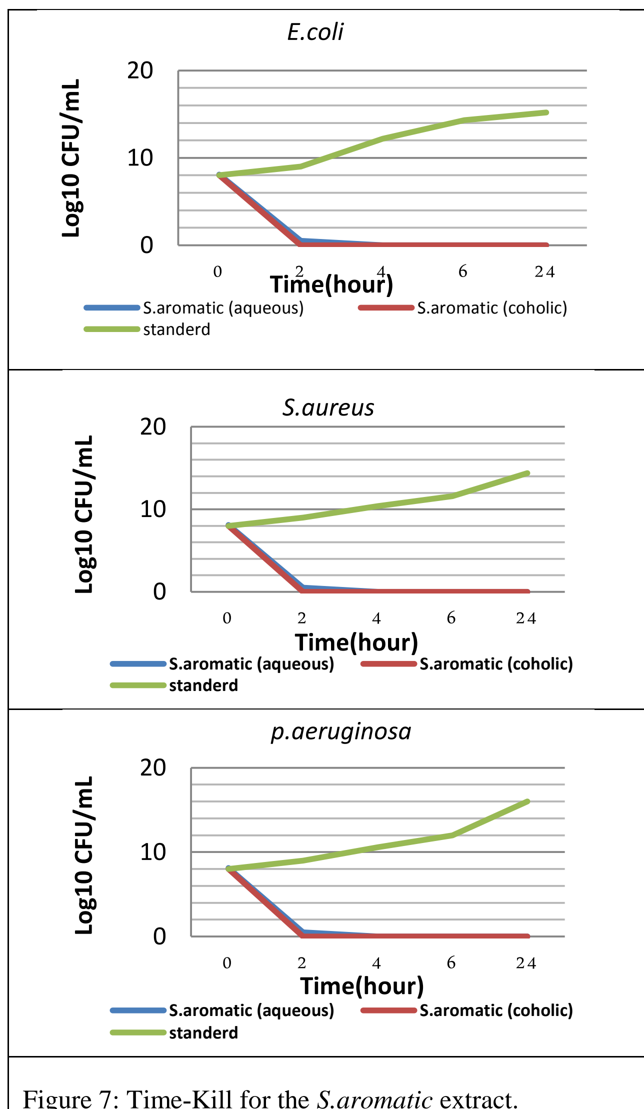


Figure 7: Time-Kill for the *S.aromatic* extract.

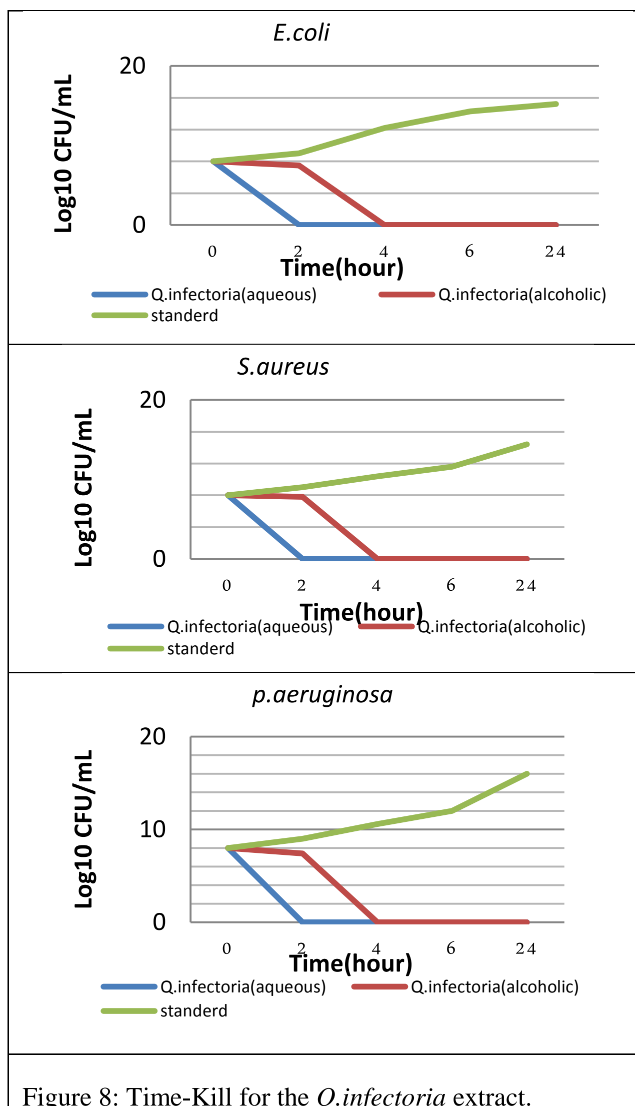


Figure 8: Time-Kill for the *Q.infectoria* extract.

These results are also evidenced through the work done by [43] this study indicates that the antioxidant effect in *Q.infectoria* is due to the phenolics_simple phenolic compounds, Phenolic acid, anthocyanins and flavonate derivatives that play an important role in counteract free radicals.

Toxicity of plant extracts:

The toxicity of plant extracts was studied on human blood by observation of red blood cell analysis on blood agar, the result show that the *S.aromatic* extract didn't analysis red blood cells (RBCs). These result are also evidenced through the work done by [39] where their study showed that the *S.aromatic* have no toxicity if the concentration was less than 1500 mg/ml. While the results of the extraction of alcohol and aqueous of the *Q.infectoria* show accumulation of red blood cells RBCs directly

after the addition of the extract and the presence of a zone of analysis in the blood agar and this is in counteract with [40] where they found that the extract of alcohol of *Q.infectoria* did not affect the red blood cells RBCs for livestock Sheep, Based on the results of the aqueous and alcohol extracts of *Q.infectoria* balls, this is considered the first study to prove the toxicity of this plant on the red blood cells of humans in Iraq. As shown in Figure (9).

Table 2: Shows the concentration of the catalase enzyme in the plant extracts and the optical density of each extract.

Q.infectoria (aqueous)	*Q.infectoria* (alcoholic)	*S.aromatic* (aqueous)	*S.aromatic* (alcoholic)	
95	410	165	210	**Catalase (U/L)**
0.230	1.115	0.394	0.530	**O.D. 490 (nm)**

*O.D. = Optical Density

* Each unit of Catalase break down 1 Micro Mall of H_2O_2 in one minute at pH = 7 at room temperature.

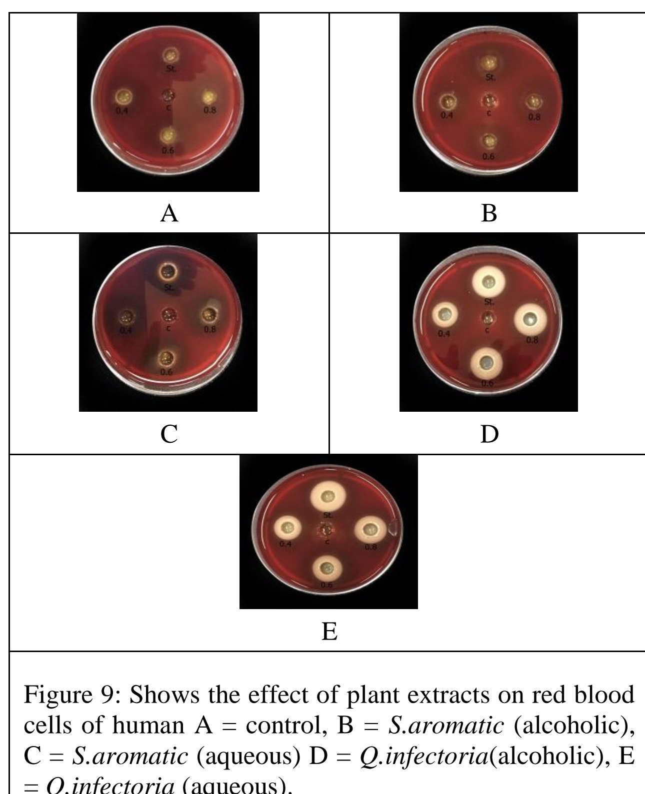


Figure 9: Shows the effect of plant extracts on red blood cells of human A = control, B = *S.aromatic* (alcoholic), C = *S.aromatic* (aqueous) D = *Q.infectoria*(alcoholic), E = *Q.infectoria* (aqueous).

Conclusions

The finding provides an insight into the usage of balles od Q.infectoria and S.aromatic in the treatment of bacterial infections caused by *E. coli ,S.aureus and P.aeroginasa* .based on the results of Q.infectoria balles extracts effects on RBCs,the treatment by Q.infectoria should be i*n vitro*.

References

[1] B. Foxman, The epidemiology of urinary tract infection, vol. 7(12), Nature Reviews Urological, 2010, pp. 53-60 .

[2] A. Ronald, The etiology of urinary tract infection: traditional and emerging pathogens, vol. 49(2), Disease a month, 2003, pp. 71-82.

[3] M. Grabe, T.E. Bjerklund, H. Botto, M. Çek, K.G. Naber, R.S. Pickard, P. Tenke, F. Wagenlehner, B. Wullt, Guidelines on Urological Infections , 2013, pp.12-49 .

[4] R. Farinaz, R. Marjan, A survey on Urinary Pathogens and their Antimicrobial Susceptibility among Patients with Significant Bacteriuria, vol. 3(4) ,Iranian Journal of Pathology, 2008, pp. 191-196.

[5] K. Amit, J. Neeraj, L.A. Madan , S. Manjeet, Antibacterial activity of some Medicinal Plants used against UTI causing Pathogens, Vol. 4, International Journal of Drug Development and Research, 2013, pp. 1-7.

[6] S. Horieh, O. Parviz, R.J. Mohammad, Z.E. Farid, A 3-Year Study Of Demographic Characteristics Of Patients With Urinary Tract Infection, Microbial Etiology, And Susceptibility Of Isolated Bacteria To Antibiotics In Shaheed Mostafa Khomeini Hospital, vol. 1(3), Iranian Journal of Pathology, 2006, pp. 99-104.

[7] E.W. Nester, D.G. Anderson, C.E. Roberts, N.P. Nancy and T.N. Martha, Microbiology a human perspective, (3rd)

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

ed. Mcgraw- Hill Higher Education , 2001, pp. 110-115.

[8] Bassam Abdul Rasool Hassan, Medicinal Plants (Importance and Uses). Vol. 3, Pharmaceut Anal Acta, 2012, pp. 1 .

[9] V. Marian, L. Dieter, M. Jan, T.D. Mark, M. Milan, and T. Joshua, Free radicals and antioxidant in normal physiological functions and human disease, The international journal of biochemistry and cell biology, 2007, vol. 39, p. 44-84.

[10] I.M. Shihata, A pharmacological study of Anagallis arvensis. M.D. Vet, Thesis, Cairo University (Cited by Al- Doori). 1951 .

[11] J. Vandepitte,; K; Engback, P. Piot, and G. Heuck, Basic Laboratory procedures in: Clinical Bacteriology. W.H.O. Switzerland. 1991 .

[12] B. S. Fabrice, D.M. Pierre, and M. F. Carl, The Potential Application of Syzygium aromaticum and Cymbopogon citratus Essential Oils as Natural Preservatives of Beef Patties, Food and Nutrition Sciences, Vol. 6, pp. 374-385.

[13] Kharma, and D.S. Hassawi, The genetic relationship and antimicrobial activity of plantago species against pathogenic bacteria. Vol. 2(3):, World Journal of Agricultural Sciences, 2006, Pp. 311-318.

[14] Clinical and laboratory standards institute Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. Vol. 27, No.3. 2007 .

[15] M. Amir, I. Darah, F. Shaida, and M. Leila, Time-kill Study of Ethyl Acetate Extract of Stinging Nettle on Bacillus subtilis subsp. spizizenii ATCC CRM-6633 Strain NRS 231, Sciencedomain international, 2015, vol. 6(1), pp. 33-40.

[16] Clinical and laboratory standards institute Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. Vol. 27, No.3. 2007 .

[17] F. Diego,; R.F. Claudia, and P.O. Wanderley, (2014). S.aromatic (Syzygium aromaticum): a precious spice., vol. 4(2), Asian Pacific Journal of Tropical Biomedicine,2014, pp. 90-96.

[18] S. Keerti,; S. Sherendra, K. Skand,; Mishra, and D. Kantishree, (2014). In vitro Antimicrobial Activity and Phytochemical Screening of Syzygium aromaticum., Vol. 4, Asian J. Res. Pharm. Sci., 2014 , pp. 12-15.

[19] M. Ikram, and F. Nowshad, Constituents of Q.infectoria infectoria., vol. 31, Planta Medica.,1977, p. 286-7.

[20] C. Wiart, and A. Kumar, Practical Handbook of Pharmacognosy. Malaysia: Pearson Education Malaysia Sdn. Bhd. 2001.

[21] Mohammad, S. Soyar and N. Mohammadreza, (2015). Prevalence and Antibiotic Susceptibility Pattern of E. coli Isolated from Urinary Tract Infection in Patients with Renal Failure Disease and Renal Transplant Recipients., vol. 14 (4), Tropical Journal of Pharmaceutical Research, 2015, pp. 649-653.

[22] M. Abubakar, Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamawa state, Nigeria., Vol. 1(1) , Journal of Clinical Medicine and Research, 2009, pp. 1-8 .

[23] Hemalatha, and P. Dhasarathan, (2010). Multi-Drug Resistant Capability of Pseudomonas aeruginosa Isolates from Nasocomal and Non-Nasacomal Sources. , Vol 2 (4), Journal of Biomedical Science, 2010, pp. 236-239 .

[24] Michael, J. Daniel, and J. James, How antibiotics kill bacteria: from targets to networks. Vol.8, Microbiology, 2010, pp.423-435.

[25] S. Barbara, Infrared Spectroscopy: Fundamentals and Applications, 2004, pp. 46-48, 72, 77.

[26] N. Suhaila, Antibacterial activity of Q.infectoria infectoria extracts against bacterial isolated from wound infection. Vol. 4, Journal of Kirkuk University, 2009, P. 20-29.

[27] E. Ayub, S. Nabi, M. Karim and M. Issa, Study on antibacterial and antioxidant

activity of Oak gall (Q.infectoria infectoria(extracts from Iran. Vol. 14, International Journal of Current Science, 2015, pp. 44-50.

[28] J.Parekh, and S.Chanda, In-vitro antimicrobial activities of extract of Launaea procumbens Rox b. (labiateae),Vitis vinifera L. (Cryperaceae). vol. 9: , A African Journal of Biomedical Research,2006, p. 89-93.

[29] B. Abu- Shanab ,G. Adwan , D. Abu-Safiya,N. Jarrar, and K. Adwan , Antibacterial activities of some plant extracts untilized in popular medicine in Plastine , vol. 28, Turkish Journal of Biology, 2004: p. 99-102.

[30] I.A. Hassan, J.A. Abdel-Barry, and S. T. Mahemmeda, The hypoglycaemic and antihyperglycaemic effect on citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. , vol. 71(2), Journal of Ethnopharmacology., 2000, pp. 325-330.

[31] M.M. Cowan,(1999).Plant products as antimicrobial agent, vol. 12(4), ClinMicrobiol Review, 1999, pp. 564-582.

[32] R. Ramanathan, C. Tan, and N. Das, (1992).cytotoxic effect of plant polyphenols and fat soluble vitamins on malignant human cultured cells, Vol. 62, Cancer letters.1992, pp. 217-224.

[33] T. Ozcan, A. Akpinar-Bayizit, L. Yilmaz-Ersan, and B. Delikanli, Phenolics in Human Health. Vol. 5, International Journal of Chemical Engineering and Applications, 2014, pp. 393-396.

[34] G. Priscila, F. Angela, C. Letícia, M. Patricia, C. Thereza, and P. Vessoni, (2009). Minimal inhibitory concentration (MIC) etermination of disinfectant and/or sterilizing agents. Vol. 45,, Brazilian Journal of Pharmaceutical Sciences., 2009, pp. 241-248.

[35] G. Philip, S. Matthew, R. E. Martin, and J. A. Rosalind, Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics, molecular systems biology, 2015, Vol. 11(3), pp.1-11.

[36] Barra, V. Coroneo, S. Dessi, P. Cabras, and A. Angioni, Chemical variability, antifungal and ntioxidant activity of Eucalyptus camaldulensis essential oil from Sardinia. Vol. 5, Natural Product Communications, 2010, pp. 329-335.

[37] K.G. Lee, and T. Shibamoto, Antioxidant property of aroma extract isolated from S.aromatic buds (Syzygium aromaticum (L.) Merr. Et Perry). ,Vol. 74, Food Chemistry, 2001, P. 443-448.

[38] R. Nidh, M. Sandhya, Sudhanshu, and M. Ekta, In vitro phytochemical screening, antioxidant and antimicrobial activity of the methanolic extract of Q.infectoria infectoria L.academic scienes, Vol. 5, International Journal of Pharmacy and Pharmaceutical Sciences, 2013, PP. 274-277.

[39] Y.A. Kabiru, J.I. Okogun, T.A. Gbodi, H.A. Makun, And E.O. Ogbadoyi, Subchronic Toxicity Studies of Methanol Extract of Eucalyptus Camaldulensis (Leaf) In Wister Rats. , Vol. 3, Iosr Journal of Pharmacy, 2012, pp. 38-45.

[40] T.H. Saba, SH.H. Isam and A.H. Manal, Identification of quantative chemical compounds of ethanolic extracts of Q.infectoria infectoria and studies its inhibitory effect in some bacteria. Vol 2 , Indian journal of research, , 2013 , p 125-128.

Copyright © 2017 Authors and Al-Mustansiriyah Journal of Science. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.