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**RESEARCH ARTICLE**

# Antimicrobial Effect of Alcoholic and Water Extracts of Thymus Vulgaris and Hibiscus sabdariffa on Different Bacteria Isolated from Raw Cow Milk.

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## *Abstract*

This study was designed to explore the effect of the ethanolic and water extract of Thymus Vulgaris and Hibiscus sabdariffa on E.coli, E coli O157 , Salmonella ,Pseudomonas, Proteus, Klebsiella, Brucella ,campylobacter and Staphylococcus . (96) Raw milk samples were collected from local diary market in Baghdad. Microorganisms were planted on muller Hinton agar. Plant extract was applied using a steers replicator and petri dishes were incubated at (37˚C) for (24 hours). The result of bacterial isolates revealed the following bacterial strain, E.coli , E coli 157, Salmonella spp, Pseudomonas spp, , Proteus, Klabsella spp , Burcella Spp , campylobacter and staphylococcus spp . The antibacterial activities were determined by measuring the diameter of the zone in mm. The result showed that high growth inhibition zones were seen with Thymus Vulgaris on Salmonella followed by E.Coli ,E.Coli O157 ,while high growth inhibition zones were seen with Hibiscus Sabdariffal on Proteus followed by Salmonella

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## 1) Introduction

Milk and dairy products is the most food group concept all over the world [1] because it contain many nutrients and provide a quick and easy way of supplying these nutrients to the diet within relatively few calories [2] otherwise milk is the ideal media for grow many bacteria [3] fresh milk from healthy cow contain few bacteria but contamination during handling can rapidly increase bacterial numbers [4] causing food poisoning [5] Synthetic antibiotics provide the main basis for the therapy of bacterial infections. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug-resistant strains of several groups of microorganisms [6]. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics. The demand for plant-based therapeutics is increasing in both developing and developed countries because of growing recognition that they are natural products, non-narcotic, and easily biodegradable, producing minimum environmental hazards, having no adverse side effects, and being easily available at affordable prices. [7]

Sorrel, H. sabdariffa L. (Family Malvaceae), a medicinal herb commonly uses to make drink and pickle, is used in folk medicine in the treatment of hypertension, liver diseases, and fever [8] . have been found to have cardioprotective[9], The aqueous extract was found to be effective against Ascaris galliavium in poultry. Also, the coloring matter of the calyces is said to be lethal toMycobacterium tuberculosis [10]. and was also found to be anticarcinogenic.[11]

Thymus Vulgaris are commonly used to treat respiratory disease(bronchitis and asthma) and has analgesic properties, antiseptic, stimulant for the circulatory system, facilitates the secretion of sweat, heart muscle tonic, prevents hardening of the arteries, treated urinary tract infection and reduce cholesterol [12]. Thyme also stimulates the memory, treatment of gums, dental pain, mouth inflammation, larynx, trachea and enters in the manufacture of toothpaste because it is perfumed the mouth, Thyme works as stomach alert, gases expulsion, prevent fermentation, helps the digestion, expel fungus from stomach and intestines, increase appetite for food because it contains Thymol which kills bacteria and parasites from stomach [13] .

These findings contribute to support and qualify the importance of screening natural products Conclusive information is, however, critical with regard to its role as an antimicrobial. The efficacy Alcoholic and Water Extracts of Thymus Vulgaris and Hibiscus sabdariffa of at( 5%, 10%, 15% and 20%) has not been tested on E.coli , E coli( 157), Salmonella spp, Pseudomonas spp, , Proteus, Klabsella spp , Burcella Spp , campylobacter and staphylococcus spp .isolated from cow raw milk .

## Materials and methods

**Sample collection:**

(96) Raw milk samples were collected from local market in Baghdad. One milliliter of each milk sample was inoculated into peptone broth and incubated at (37°C) for (18–24) hours. After incubation, about 100µl of the inoculated peptone broth were sub-cultured onto plates of blood agar , Nutrient agar, Maconky agar, Eosin methylene blue (E.M.B),Sorbitol- Macconcy agar with cefixime tellurite ,Manitol salt agar , trypticase soya agar, Salmonella Shigella Agar (S.S) agar, and the biochemical properties were tested depending on the method of [14] bio-chemical is (Sulfur Indole Motility Test (SIM), Triple Sugar Iron (TSI), Simmon Citrate Test (SC), Urease and Methyl Red -Voges Proskauer Test (MR/VP). Special latex for E.Coli (O157). Pure culture of bacterial isolates were identified and confirmed diagnosis by morphological features and biochemical tests according to [15].

**Preparation of ethanolic and water Extracts**

Thymus Vulgaris and Hibiscus sabdariffa Plants were blended in an electrical blender (sharp, Japan) until obtained final powder , weight (150 gm) of plant in flask and added (450 ml) of alcohol solution (70%) Ethanol, put the flask in freezer (-20ċ ) for (9) days after that put it in on a magnetic stirrer for (20) minutes, filter the extract by gauze then filter paper (240mm) in size then put in oven 37ċ for 3days to preparation of dose concentration as table (1).

**Antibiotic Sensitivity test :**

Standard Ampicillin (AM10µg/disc), Cloxacillin (CX, 1µg/disc), Erythromycin(E, 10 µg/disc), Doxycycline (DO, 30µg/disc), Cephalexin(CL, 30 mcg/disc) , Trimethoprim (TMP 25µg/disc),Azethromycin (AZM), Rifampin (RA 5 µg/disc) and Ciprofloxacin (5 µg/disc) . were used for comparison of the antibacterial activity.antibacterial susceptibility of the test samples was performed on Mueller-Hinton agar by disc diffusion method [16]The antibacterial activities were determined by measuring the diameter of the zone in mm.

## Results and discussion:

The result showed that (80) milk samples contaminated with E.coli (83.9%) fallowed by E.coli O157 (27) sample (28.1%) then pseudomonas (25) sample (26%) and campylobacter (22) sample (22.9%), Staphylococcus (17) sample (17.7%) ,salmonella (11) sample (11.45%) , Klebsella (9) Sample (9.38 %) , Brucella (7) sample (7.29%) and Proteus(5) sample (5.2%) respectively . some samples expressed mixed bacterial isolates and other showed pure single bacterial colonies.

The result showed high growth inhibition zones were seen with Hibiscus Sabdariffal on Proteus followed by Salmonella., inhibition zones were seen within each isolate started at the (5%) level of sorrel concentration of alcoholic extract ranged from (9 mm) on Staphylococcus to (22 mm) on Proteus while the greatest inhibition zones seen at the (20%) level of sorrel concentration of watery extract ranged from (22mm) on Campylobacter to (32mm) on Proteus .this experiment showed that the Watery extract is the most potent in all concentration. these result agreed with [17] also [18] who found that Hibiscus extracts have the potential to be a natural alternative to

antimicrobials currently used in foods against E.coli O157H7 ,S aureus, and L. monocytogenes . The antimicrobial activity due to flavonoids, they have the ability to form a combined complex with bacterial cell walls.[19] Also, with the number of hydroxyl groups present on the phenolic ring which increased hydroxylation, and with increased hydroxylation there will be increased antimicrobial activity [20] the antimicrobial action mechanism of action may be by inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the bacterial cells[19].

**Table -1**

| Volume of solvent/ ml (water &ethanol) | Weight of dried extracts (g) | Concentration of extract |
|---|---|---|
| **10** | **0.5** | **5%** |
| **10** | **1** | **10%** |
| **10** | **1.5** | **15%** |
| **10** | **2** | **20%** |

**Table: 2**

| Hibiscus Sabdariffal | Alcoholic | | | | Watery | | | |
|---|---|---|---|---|---|---|---|---|
| | 5% | 10% | 15% | 20% | 5% | 10% | 15% | 20% |
| **E. Coli** | **17** | **20** | **26** | **30** | **18** | **21** | **27** | **31** |
| **E .Coli O157** | **16** | **18** | **24** | **28** | **19** | **26** | **28** | **30** |
| **Pseudomonas** | **11** | **14** | **22** | **25** | **13** | **21** | **24** | **26** |
| **Salmonella** | **18** | **20** | **21** | **23** | **13** | **18** | **26** | **27** |
| **Campylobacter** | **12** | **14** | **19** | **22** | **16** | **18** | **20** | **23** |
| **Brucella** | **16** | **17** | **20** | **25** | **18** | **19** | **22** | **24** |
| **Staphylococcus** | **9** | **12** | **18** | **24** | **10** | **14** | **22** | **25** |
| **Klebsella** | **16** | **17** | **18** | **22** | **16** | **18** | **22** | **25** |
| **Proteus** | **22** | **24** | **26** | **28** | **26** | **25** | **28** | **32** |

**Table (3) showed that high growth inhibition zones were seen with Thymus Vulgaris on Salmonella followed by E.coli, E.coli O157, Results showed the antimicrobial activity appeared to be more marked with the increase the** concentration of either both extracts, and the activity of alcoholic extract more than the watery extract The inhibition zone started with(8 mm) in(5%) concentration of watery extracts on Klebsella, while the most antimicrobial effect of Thymus Vulgaris showed in (20%) concentration of alcoholic extract which gave ( 28 mm) inhibition zone on Salmonella ,these result agreed with [13] who reported inhibitor effects of Thymus Vulgaris against nine strains of gram-negative bacteria and six strains of gram-positive bacteria Escherichia coli O157:H7

was the most sensitive species, this effect may be due to Thymus essential oil [21]. flavonoids, the most important Eridicytol, Luteolin, and Apigenin [22], Tannins and Saponins [23]

**Table:3**

| Thymus vulgarisl | Alcoholic | | | | Watery | | | |
|---|---|---|---|---|---|---|---|---|
| | 5% | 10% | %15 | 20% | 5% | 10% | 15% | 20% |
| E. coli | 12 | 14 | 17 | 25 | 14 | 16 | 20 | 23 |
| E.coli O157 | 14 | 15 | 21 | 26 | 7 | 15 | 20 | 26 |
| Salmonella | 19 | 22 | 23 | 28 | 16 | 18 | 23 | 27 |
| Pseudomonas | 10 | 12 | 15 | 22 | 11 | 13 | 17 | 21 |
| campylobacter | 14 | 16 | 18 | 23 | 13 | 17 | 20 | 22 |
| Brucellas | 9 | 13 | 16 | 20 | 9 | 15 | 18 | 20 |
| Klebsella | 10 | 13 | 16 | 18 | 8 | 11 | 16 | 19 |
| Proteus | 10 | 14 | 17 | 20 | 13 | 16 | 18 | 19 |
| Staphylococcus | R | 12 | 18 | 20 | 11 | 13 | 15 | 19 |

**Table: 4**

| Bacteria | C X | D O | AZM | TMP | E | R A | CL | A M | CRO |
|---|---|---|---|---|---|---|---|---|---|
| pseudomonas | R | 5 | 23 | 5 | 20 | R | R | R | R |
| salmonella | 2 | R | 15 | 5 | 13 | 5 | 3 | 2 | 12 |
| E.coli | R | 9 | 20 | R | 10 | R | 14 | R | R |
| campylobacter | 2 | 6 | 3 | 20 | 4 | 1 | R | 7 | 6 |
| E .Coli O157 | 2 | 9 | 20 | 5 | 8 | R | 23 | R | R |
| Brucela | 3 | R | 15 | R | 5 | R | 5 | 4 | 4 |
| Klebsella | R | 6 | 16 | 12 | R | 3 | R | 2 | R |
| Staphylococcus | R | 3 | 12 | R | 4 | 3 | 5 | R | R |
| Proteus | R | 5 | 23 | 20 | R | R | R | 5 | R |

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