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# The efficacy of Carica papaya leaf extract against some bacteria which isolated from cow milk

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***Abstract*---**The advent of science to the search for antibiotics principally depends on medicinal plants as raw materials. Pathogenic bacteria isolated from cow milk represent play a major role in mastitis and are not easy to treat even with the use of a wide range of antibacterial agents. Finding alternative substances that may eliminate those bacteria with fewer side effects especially if they are derived from natural sources. Here, papaya leaf was collected, air-dried, powdered and extracts (PLE) of tow solvents; ethanol (EPLE) and aqueous (EPLA) were used to evaluate their Mueller-Hinton based inhibition activities against Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus (isolated from milk of local cows in the current work). The results showed that the ethanol extract of papaya leaves (EPLE) was highly effective in decreasing the growth of Klebsiella pneumoniae and Staphylococcus aureus, while did not show any activity in inhibiting of E. coli. Compared with the aqueous extract of papaya leaves (EPLA), which did not record any inhibition of the growth of bacteria isolated for the study only in its high concentration (80%) for each (E. coli and Klebsiella pneumoniae).

***Keywords*---**Bacterial mastitis, Papaya extracts, GC mass of papaya leaf.

## Introduction

Mastitis is an infectious disease of the mammary glands of small ruminants that can cause considerable or complete damage to the udder, cause the body to gain weight, and slow the growth rate of the progeny (1). As a result, economic wastes arise from the loss of animals and treatment components. Mastitis, both clinical

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and subclinical refers to the permanent occlusion of milk ducts caused by severe inflammatory transient symptoms (2,3). Changes in the mammalian gland that is unpleasant, poisonous, and bacteriological Maladministration, for example, poor grooming and teat accidents are regarded foreign, allowing infections to enter more easily. In mastitis, pathogenic bacteria can only be seen in lab experiments. Mastitis is one of the most serious subclinical illnesses, and it is thought to be a constant source of infection for the entire flock and ecology (4, 5).

As a result, udder pathogens must protect not just animals but also the general populace in order to be effective. Early detection or avoidance is recommended. Mastitis in dairy cattle is caused by a variety of factors. Infectious agents caused by Staphylococcus aureus, Staphylococcus spp., and other bacteria (6, 7, 8). Cattle slaughter (39.4%) and a decrease in milk output due to subclinical and clinical mastitis had the greatest economic impact (32, 3 % and 18, 2 % respectively) (5). Using actual mastitis indicators, the decrease in milk supply due to mastitis (77.7%) and the dumping of milk (14.0%) were the two most relevant factors. The decrease in milk output was 27.2 % higher than expected. However, more detailed calculations revealed that the financial impact was worth 91, 552, 69 dollars (9, 10). Antibiotic treatments, milk disposal, and animal deaths account for the majority of the financial costs. Antibiotic use causes crises in a variety of ways, one of which is by introducing antibacterial resistance into the system and causing adverse effects like as a decline in milk output (11). Finding alternative natural sources of antibacterial chemicals could thereby improve the overall wellbeing of animal husbandry and, in turn, global economics. In this study, Mueller-Hinton-II based inhibitory activities against Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae (isolated from milk of local cows) were evaluated using papaya leaf extracts with two solvents: ethanol (EPLE) and water (EPLA).

## Materials and Methods

### Sample collection

In Musayyab / AL- Hella, I collected leaf plants from private farms for papaya production. The leaves were then washed in a continuous stream of distilled water for 5 minutes before being dried aerobically. Using a grinding machine, the dry samples were crushed into a powder and sieved. Samples were stored at 4°C in opaque glass containers until extraction.

### Preparation of extract

- After drying the papaya leaves, they were crushed well after removing the solid parts to obtain a powder. The (50) g of powder was soaked (500) ml of distilled water in a 500 ml beaker for (4-7) days. After shaking the extract for an hour on a continuous shaker. The extract was filtered using a Whitman No.2 filter paper, distributed on sterile dishes, and the extract was dried in an oven for two weeks.
- (50) G of leaves powder with (320) ml of ethanol alcohol and (150) ml of distilled water. Boiled the preparation solution for (30) min and keep it in a

place away from light for (4-7) days and then filtrated by Whitman No.2. Put it in sterilized dishes inside the drying oven for two weeks. (12)

**Isolation of bacteria from milk**

Milk samples were taken from cows on a dairy farm in the Iraqi region of Al-Diwaniyah. This study focused on E. coli, K. pneumonia, and S. aureus bacteria. Each bacterium was cultured on nutrient agar for (18 – 24) hours at 37°C to create colonies, which were then placed in sterile physiological saline containing tubes and vortex completely. The turbidity of each bacterium was then determined using a 0.5 McFarland reference solution (containing roughly 1.5 108 CFU/mL).

**Anti-bacterial activity of the papaya extracts**

The test was performed using Mueller Hinton II plates and the well-diffusion method, as recommended by the National Committee for Clinical Laboratory Standards [CLSI]. Three plates with 5mm diameter wells in the medium were made for each solvent. A sterile cotton swab was used to streak the plate medium. After that, 5mL of each extract was poured into each well. For 24 hours, the Para film-sealed plates were held at 37°C. After that, the inhibitory zones were measured (mm).

**GC-MS system:**

GC-MS analysis was performed at the CAC Iraq laboratory in Baghdad, utilizing an Agilent USA model 6890 combined with a mass spectrometer, model 5973N, and HP-5MS. Capillary column having a 5 percent methyl phenyl siloxane static phase (length 30 meters, inner diameter 0.25 m, and resident layer thickness 0.25 m). The energy of ionization is 70 electron volts. Data was analyzed through February 2022, with the library data base serving as an additional tool for confirming compound identity.

**Results**

The results showed that the ethanol extract of papaya leaves (EPLE) was highly effective in decreasing the growth of Klebsiella pneumoniae and Staphylococcus aureus, while did not show any activity in inhibiting of E. coli (table3-1), figure 1. Compared with the aqueous extract of papaya leaves (EPLA), which did not record any inhibition of the growth of bacteria isolated for the study only in its high concentration (80%) for each (E. coli and Klebsiella pneumoniae).

Table 1
Inhibition zones of papaya leaf extract

| Organism | Inhibition zone of EPLE | | | Inhibition zone of EPLA | | |
|---|---|---|---|---|---|---|
| | 20gm/ml | 40gm/ml | 80gm/ml | 20gm/ml | 40gm/ml | 80gm/ml |
| E. coli | - | - | - | - | - | ± 1 |
| Klebsiella pneumonia | ± 1.5 | ± 1.7 | ± 2 | - | ± 0.1 | ± 0.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Staph. Aureus | ± 1 | ± 1.4 | ± 1.6 | - | - | - |

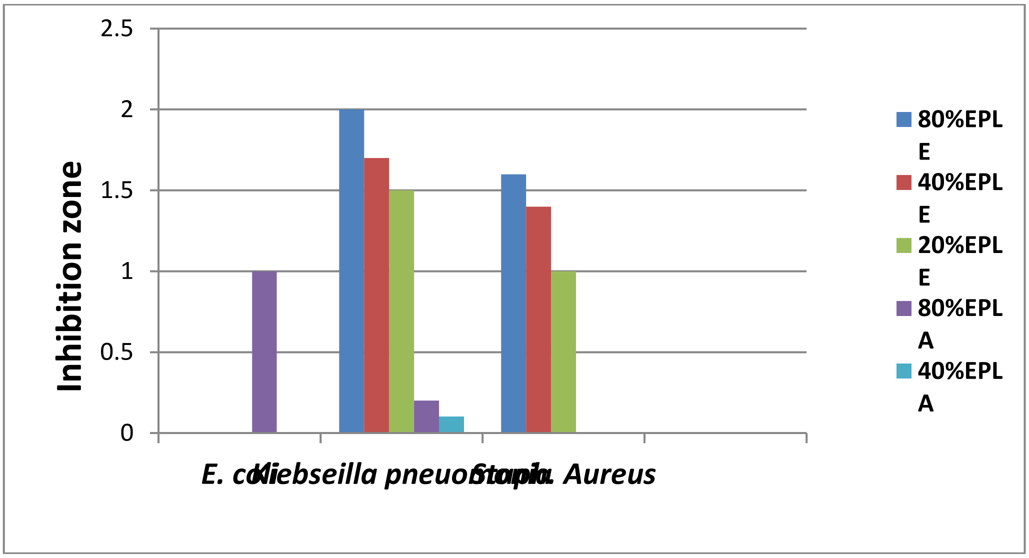


Figure 1. Inhibition zones of papaya leaf extract

The analysis of extracts using GC MASS technique also proved that there are effective compounds in papaya leaves according to the solvent used as shown in Table (2 ,3) and Figure (2, 3).

Table 2
Phytochemicals discovered by GC-MS in C. papaya ethanol leaf extract

| Peak No | Name | Formula | Retention time/MN | Area% | Molecular Weight |
|---|---|---|---|---|---|
| 1 | D-Limonene | C10H16 | 17.621 | 1.50 | 136.23 |
| 2 | Eicosane | C20H42 | 46.878 | 1.59 | 282.5 |
| 2 | Heptadecane | C17H36 | 46.878 | 1.59 | 240.5 |
| 3 | Hexanedioic acid, bis (2-ethylhex... | C22H42O4 | 52.473 | 32.53 | 370.56 |
| 3 | Z-5-Nonadecene | C19H39 | 53.056 | 2.10 | 266.5 |
| 4 | 2-Pentadecanone | C15H30O | 59.343 | 2.06 | 226.40 |
| 8 | Koiganal I | C18H34O | 60.480 | 3.21 | 266.46 |
| 10 | (+)-trans-. alpha. -himachalene | C15H24 | 61.080 | 5.31 | 204.35 |
| 11 | Cyclotetracosane | C24H48 | 61.640 | 7.01 | 336.6 |
| 13 | Thiosulfuric acid (H2S2O3) | H2S2O3 | 62.206 | 4.74 | 114.14 |
| 13 | Octadecanoic acid | C18H36O2 | 62.206 | 4.74 | 284.5 |
| 14 | 14-. BETA. -H-PREGNA | C21H36 | 62.206 | 4.74 | 288.5 |
| 14 | Neophytadiene | C2OH38 | 22.589 | 3.216 | 278.5 |
| 14 | Tocopherol ( vit E) | C29H50O2 | 33.851 | 12.431 | 430.7 |

Table (2) and figure (2) showed the presence of twelve plant components in the ethanolic papaya leaf extract. Through comparative examination, the main components present in the papaya leaf extract of the local variety in terms of their relative abundance were Hexadecanoic acid, Cyclotetracosane, -alpha.-himachalene, Thiosulfuric acid, Octadecanoic acidandBETA.-HPREGNA,KoiganalI,Z5Nonadecene,2Pentadecanone,Heptadecane,Eicosane,D-Limonen(32.53,7.01,5.31, 3.21,4.74,4.74,4.74,2.10, 2.06, 1.59,1.591.50)% relative abundance respectively.

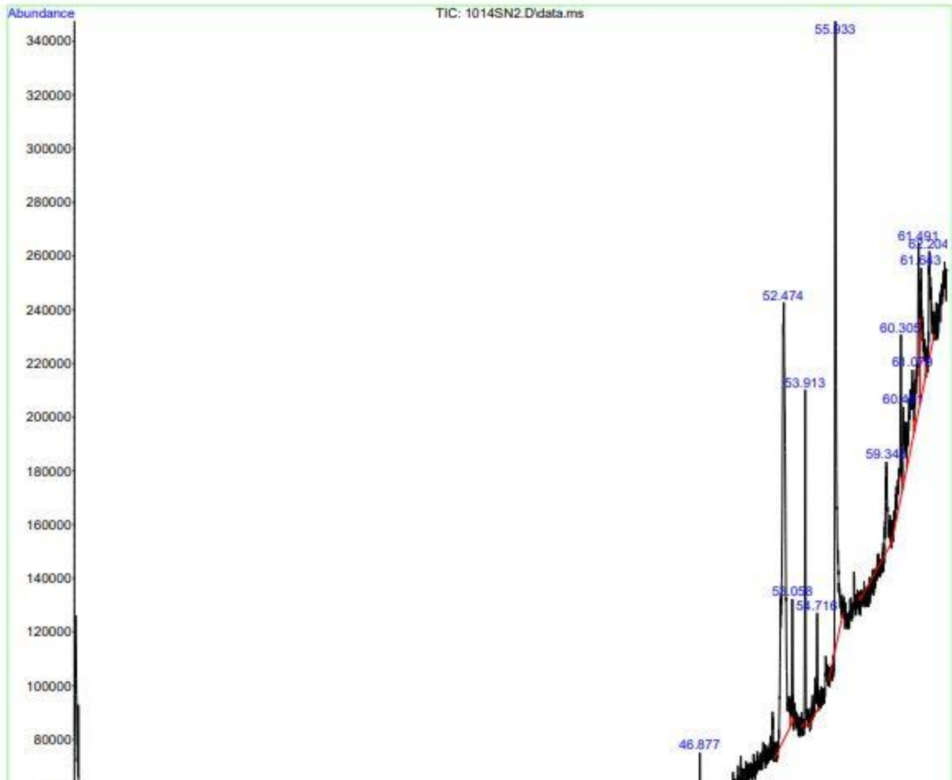


Figure 2. Showed the presence plant components in the ethanolic papaya leaf extract

Table 3
Phytochemicals identified in aqueous leaf extract of C. papaya by GC-MS

| Peak.NO | Name | Formula | Retention Time/mn | Area % | Molecular Weight |
|---|---|---|---|---|---|
| 1 | 2-Pentanone, 4- | (CH3)2 | 9.460 | 3.71 | 116.16 |

| | | | | | |
|---|---|---|---|---|---|
| | hydroxy-4-methyl- | C(OH)CH2COCH3 | | | |
| 2 | Benzyl nitrile | C6H5CH2CN | 22.890 | 1.62 | 117.15 |
| 3 | 2-Methoxy-4-vinylphenol | C9H10O2 | 31.092 | 0.96 | 150.177 |
| 4 | Benzene, (isothiocyanatomethyl) | C10H8N2S2 | 33.447 | 0.58 | 220.3 |
| 5 | Hexadecane | C16H34 | 43.083 | 0.95 | 226.448 |
| 6 | Neophytadiene | C20H38 | 51.741 | 0.34 | 278.5 |
| 7 | Hexanedioic acid, bis(2-ethylhex | C22H42O4 | 52.599 | 61.95 | 370.6 |
| 8 | n-Hexadecanoic acid | C16H32O2 | 55.936 | 5.31 | 256.42 |
| 9 | 2-Hexadecen-1-ol, 3,7,11,15-tetr | C20H40O | 60.612 | 1.93 | 296.5 |
| 10 | 2H-Pyrrol-2-one, 1, 5-dihydro-4-m | C5H7NO | 61.635 | 3.22 | 97.12 |
| 11 | 3,6,6-Trimethylundecane-2,5,10-t | C14H24O3 | 63.201 | 1.48 | 240.34 |

The presence of eleven plant components in the aqueous papaya leaf extract was shown in table (3) and figure (3). Hexane dioic acid, bis (2-ethylhex, n-Hexadecenoic acid, 2-Pentanone, 4-hydroxy-4-methyl-,2H-Pyrrol-2-one, 1, 5-dihydro-4-m, 2-Hexadecen-1-ol, 3,7,11,15-tetr, Benzyl nitrile,3,6,6Trimethylundecane2,5,10t,2Methoxy4vinylphenol, Hexadecane, Benzene, (isothiocyanatomethyl), Neophytadiene (61.95,5.31,3.71,3,22,1.93,1.62,1.48,0.96,0.95,0.58,0.34)% relative abundance respectively.

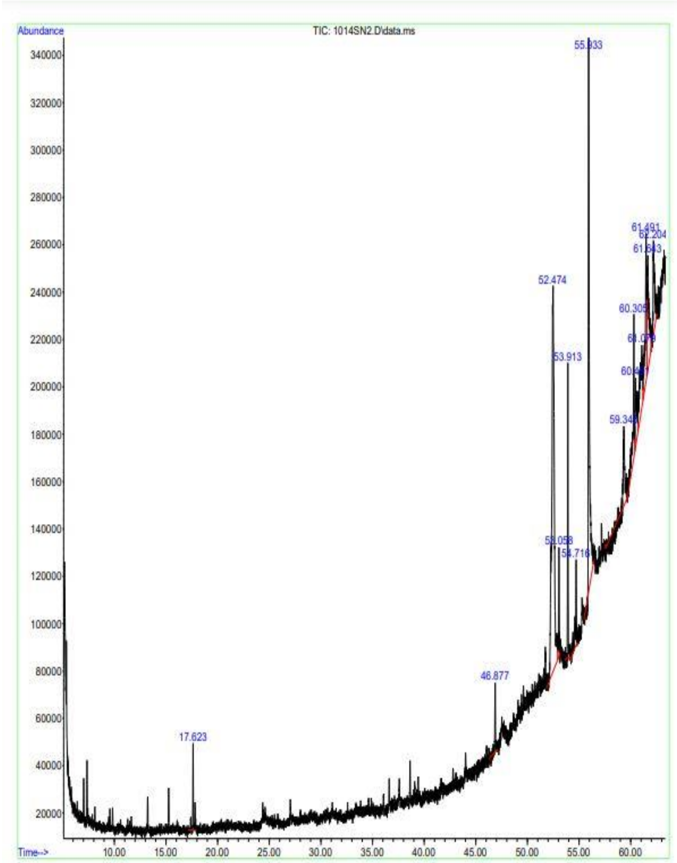


Figure 3. showed the presence plant components in the ethanolic papaya leaf extract

**Discussion**

Mastitis affects the dairy industry, which has an impact on milk yields and consistency. Many species have been connected to mastitis instances, with E. coli, Klebsiella spp., Streptococcus dysgalactiae, and Staphylococcus aureus being the most specific bacteria linked to clinical mastitis. I identifying the underlying cause microorganism enables for accurate diagnosis and proactive mastitis prevention with the use of appropriate antimicrobials. For the prevention and control of mastitis and other bacterial diseases affecting the dairy industry in addition, there is antimicrobial treatments are increasingly being used in dairy farms. Antimicrobial resistance exists in microorganisms capable of counteracting the effects of previously effective antibacterial agents. This is still a serious public health issue in Europe, as well as one of the most pressing environmental and food security concerns in the global economy (13, 14, 15, 16). Finding natural alternatives may aid in enhancing the Figure 2&3: Papaya leaves screened solvents with antibacterial activity Antibiotic resistance problem and reducing the spread of this health concern with successful treatment results. The current study found that papaya leaf extract contains a number of useful and vital chemicals, which varied in terms of their presence and availability depending on the type.

Papaya leaf extract has the highest levels of Heptatriene, 15-octadecatrienoate, Tocopherol and Hexadecanoic acid were found to be important plant components in papaya leaf extract in the current investigation. Which has antioxidant, antibacterial, and anticancer properties (17, 18) the antibacterial and antioxidant agent's n-hexadecanoic acid which were found in significant levels in papaya leaf extract, were probable hypocholesterolemia and antihypertensive agents (19). The ethanol extracts of this plant were used in most pharmacological research, and it was discovered to have anti-stress, apoptogenic, anti-hypoxic, immune-stimulatory, anticancer, cytoprotective, radioprotective, anti-hemolytic, anti-inflammatory, and wound healing potential (20-21). However, the pharmacological effects of the plant's leaf extracts derived by semi-polar solvent extraction have been thoroughly explored. The results for antibacterial activity of Carica papaya leaf extract are shown in Table 1 and Figure 1.

It was discovered that both ethanol and aqueous extracts had varying degrees of antibacterial activity, with ethanol extract having the highest activity against Klebsiella pneumoniae and Staph aureus, but no activity against E. coli. With increasing extract concentrations, both ethanol and aqueous extracts showed an increasing zone of inhibition against Klebsiella pneumoniae. Al-Bayati and Sulaiman investigated the effect of the extraction solvent on the extract's inhibitory capacity against the test organism (2008). It's interesting to note that the fact that ethanol extracts inhibited bacteria more than water extracts suggests that the solvent system influences the bioactive component's solubility and antibacterial activity. According to Anibijuwon and UdezeDepartment.2009, the fact that the extracts were efficacious against Gram-negative and Gram-positive bacteria suggests a broad spectrum of activity. Phytochemicals were examined in the high-quality Carica leaf extract. It was determined that papaya contained bioactive chemicals. The presence of chemicals in both extracts is consistent with the hypothesis. While the absence of anthraquinones in the leaves found in this study contradicts Omidiwura's (2017) findings, it agrees with Ajani et al. (2013) and Doughari's research (2006). Carica papaya's antibacterial effects are aided by some of these compounds, which are known to be physiologically active. Plants produce phytochemicals during their primary or secondary metabolism; but, as stated by, proof of their potential health effects has yet to be established (Nwofia et al., 2012). These chemicals are recognized to be physiologically active, which helps Carica papaya's antibacterial properties (22-27).

## Conclusions

The study's findings indicated that Carica papaya has the following bioactive constituents: glycosides, saponins, tannins, steroids, and alkaloids, all of which contribute to its antibacterial properties. The activity was influenced by types of solvents. Ethanol extracts is more activity than water against certain bacteria. The activity could inhibit Klebsiella pneumonia, staph aureus, well with ethanolic papaya extracts (EPLE). The present study presents promising compounds for treating mastitis caused by these bacteria; however, clinical trials showed be performed to estimate their therapeutic doses, safety, and effectiveness.

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