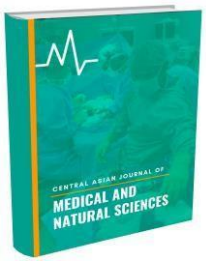

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*Article*

# The Synergistic Effect of *Cordia Myxa* and Bioemulsifier Extracted from *Saccharomyces Cerevisiae* Against Pathogenic Bacteria

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**Abstract:** Twenty-five pathogenic bacterial isolates were obtained from different isolation sources. Most of the isolates showed resistance to most antibiotics. Five isolates of baker's yeast of different origins were collected from local markets. Isolate Pa was selected as the most efficient isolate in producing the bioemulsifier, with an emulsifying efficiency of E24 = 81.8%. The method of applying the microtiter plate was adopted to test the inhibitory activity. The bioemulsion showed inhibitory activity against six selected isolates at MIC=80.82mg/ml. Both extracts from *C. myxa* contain phenols, flavonoids, and glycosides, while the water extract without the alcoholic extract lacks alkaloids and a small percentage of saponin. The water extract showed inhibitory activity against S. *Typhi, Shigella sonnei, P. aeruginosa, S. aureus, and K. pneumoniae* at (MIC) = 250mg/ml, And at MIC=62.5mg/ml towards *E. coli*. The alcoholic extract showed inhibitory activity against *S. typhi, Shigella sonnei, K. pneumoniae,* and *E. coli* isolates at MIC=62.5mg/ml, While it showed inhibitory activity against *P. aeruginosa* and *S. aureus* isolates at MIC=125mg/ml. The Boardchequer method was adopted to study the synergistic relationship and determine the FIC. The study showed a synergistic relationship between the bioemulsifier and the alcoholic extract with a fractional inhibitory concentration (FIC) = 0.267 towards isolates of *S. typhi, Shigella sonnei, K. pneumonia*, and *E. coli*, While the FIC = 0.151 towards *P. aeruginosa* and *S. aureus* isolates.

**Keywords:** *Cordia Myxa*, *Saccharomyces Cerevisiae*, Bioemulsifier

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## 1. Introduction

Antibiotic resistance is a major global health problem. This is due to bacteria developing resistance to antibiotics. This resistance reduces the effectiveness of antibiotics, Which makes treatment for the infection more difficult; this increases the risk of complications from the infection and can lead to more extended stays in the hospital, as well as higher medical costs and increased mortality rates (Renata et al., 2022). Resistance of bacteria to antibiotics has led to an increase in the spread of these bacterial strains. This reduces treatment options as common infections may become untreatable. Multi drug-resistant bacteria have become a public health threat (Yagul et al., 2023).

The resistance of bacteria to a vast range of antibiotics is one of the causes that propel researchers to inspect solutions to confront them. The most notable of these solutions are the effectual compounds microorganisms make in the face of other organisms, Such as bioemulsions. One of the most critical bioemulsifiers is *Saccharomyces Cerevisiae* emulsifier. It is the outer constituent of the yeast wall and is made distinctive by a complex constituent

of protein and carbohydrates(Abdel Hamid,.2020 and H.G.H. Rasheed and N.H. Haydar., 2023). Baker's yeast emulsion has the unique capability to demolish the cell walls of other microorganisms. In addition to its capability to lessen the development of biofilms(Saleh et al.,2020). This was not limited only to the energetic compounds made by microorganisms. Instead, researchers also have recourse to plant extracts; among them are *Cordia Myxa* extracts, which aroused the curiosity of researchers because they contain active compounds such as phenols and alkaloids, as well as mucilage, which makes them have antibacterial Features(Amal, et al., 2023).

The study aimed to find therapeutic options that perform similarly to antibiotics, To make less human utilization of antibiotics and thus lessen the risk of pervasion bacteria resistance.

## 2. Materials and Methods

**Collecting bacterial isolates and identifying them:**

Twenty-five bacterial isolates were collected from dissimilar isolation sources. A vitek II device was used to diagnose it.

**Antibiotic susceptibility of the isolates:**

The sensitivity of the isolates to antibiotics was tried out by applying the Kirby-Bauer Disk Diffusion Susceptibility Test technique. The sensitivity and resistance of the isolates were determined by contrasting the test results with what was declared in the CLSI (2023) (Mahon and Lehman., 2019).

**Collection and diagnosis of yeast:**

It relied upon five isolates of different origins obtained from local markets. They were diagnosed Depending on the vitek II.

**Bio-emulation output:**

Cooper's medium was used to produce the bioemulsion. Yeast was added to the medium after activation. Incubate the medium for 72 hours in a shaking incubator at 30°C. The cells were then separated by centrifugation at 10,000 rpm at 4°C. The cells were then suspended in a PBS buffer (Saleh et al.,2020).

**Bio-emulation extraction:**

The 100°C heating method followed by Li et al (2019) was used for bio-emulsion extraction. After heating, centrifugation was performed at a speed not exceeding 3000 rpm. The supernatant, which represents the bio-emulsion, was taken after separation.

**Investigating which yeast is most efficacious in producing bio-emulsions:**

The method of measuring E24 emulsifying activity was adopted by Alcantara et al (2014) in selecting the yeast that produces the most bio-emulsifier among different species.

**Calculating the concentration of carbohydrates and proteins for the bioemulsion:**

Dubois et al (1956) method was applied to calculate the carbohydrate concentration, and Bradford's (1976) method was applied to calculate the protein concentration of the emulsion.

**Gather and grind *C. myxa*:**

*C. myxa* was gathered in dried form from the market; the fruits were ground and stored at 4°C until use.

**Alcoholic extract:**

"The alcoholic extract was prepared according to Murad and Karbon (2020); this is done by mixing 400 ml of 85% alcohol with 100 g of crushed *C. myxa*. Leave at four °C for overnight with continuous stirring. Use gauze to get rid of impurities. Dry the extract using a rotary evaporator. Keep the extract at four °C until use."

**Water extract:**

The technique of Larki et al (2020) was adopted. Mix 100 g of crushed *C. myxa* with 800 ml of D.W. and leave overnight with constant stirring at 25°C. Filter the extract from impurities, then dry the extract using a rotary evaporator. Keep the extract at four °C until use.

**Investigate *C. myxa* extracts on some efficacious compounds:**

**Detecting alkaloids:**

Alkaloids were disclosed for the extracts following the technique described by Kancherla et al (2019). Dissolve the extract with 1% hydrochloric acid. Then, some drops of Mayer's reagent were added to the extract. The presence of alkaloids plays a role in forming a white layer at the bottom.

**Detecting Saponin:**

Prepare 5 ml of the e extract and add drops of sodium carbonate solution. Leave the tube for 5 minutes after shaking it vehemently. The foam forming in the higher layer indicates the existence of saponins(Kancherla et al.,2019).

**Detecting Glycoside:**

Keller-Kiliani test: Add 1 ml of acetic acid at a concentration of 1% to the extract, Right after that, Add drops of 2% concentration ferric chloride to the extract. Followed by the gradual addition of 2 ml of concentrated sulfuric acid. An indication of the presence of glycosides is the formation of a reddish-brown ring(Kancherla et al.,2019).

**Detecting phenolic:**

Ferric chloride test: 2 ml of 5% neutral ferric chloride solution was added to 1 ml of extract. The dark blue color indicates the presence of phenolic compounds (Kancherla et al.,2019).

**Detecting flavonoids:**

Alkaline reagent test: Two to three drops of sodium hydroxide were added to 2 ml of extract. Initially, A deep yellow color appeared but gradually became colorless by adding a few drops of dilute hydrochloric acid. Which indicates the presence of flavonoids (Kancherla et al.,2019).

**The Inhibitory activity against pathogenic bacteria**

The method described by Elshikh et al (2016) was adopted, based on microtiter plates, by testing the inhibitory activity of the extracts against six out of 25 bacterial isolates that showed the most antibiotic resistance. In addition to adopting Resazurin stain in determining the MIC of bio-emulsified extracts and water and alcoholic *C. myxa* extracts.

**Synergistic effect of Mannoprotein and plant extraction**

The method described by Aziz (2023) was adopted to determine the synergistic effect between the bioemulsifier and *C. myxa* fruit extracts against pathogenic bacteria using the Checkerboard method. Through this, it is possible to decide on the MIC between two antibodies and their relationship type, whether synergistic or non-synergistic. This is done by calculating the fractional inhibitory concentration (FIC) by applying the following equation:

$$\mathbf{FIC} = \frac{\mathbf{A}}{\mathbf{MICA} - \mathbf{A}} + \frac{\mathbf{B}}{\mathbf{MIC} - \mathbf{B}} = \sum \mathbf{FIC} = \mathbf{FICA} + \mathbf{FICB}$$

## 3. Results and Discussion

**Identification of bacterial isolates:**

Table-1 shows the types and number of isolates and the various sources of their isolation.

**Table-1:** The number of identification isolates, their types, and isolation

| Types of isolates | isolation sources | Number of bacterial isolates |
|---|---|---|
| *Acinetobacter baumannii* | Burns | 1 |
| *Burkholderia cepacia* | Burns | 2 |
| *Escherichia coli* | Urine (UTI) | 1 |
| *klebsiella pneumoniae* | Diabetic foot | 1 |
| *klebsiella pneumoniae* | Burns | 2 |
| *klebsiella pneumoniae* | Urine | 1 |
| *Proteus mirabilis* | Diabetic foot | 2 |
| *Pseudomonas aeruginosa* | Burns | 3 |
| *Pseudomonas aeruginosa* | Urine | 1 |
| *Salmonella typhi* | Blood | 3 |
| *Shigella sonnei* | Stool (Diarrhea) | 2 |
| *Staphylococcus aureus* | Burns | 2 |
| *Staphylococcus aureus* | Urine | 1 |
| *Staphylococcus aureus* | Blood | 2 |
| *Streptococcus pneumoniae* | Sputum | 1 |

**Sensitivity of isolates to antibiotics:**

Table-2 shows the resistance and sensitivity of bacterial isolates to antibiotics. Most isolates showed 94.7% resistance to Nafcillin and Azithromycin. At the same time, most isolates showed sensitivity to the antibiotics Imipenem and Meropenem.

Table-2: **Rate of resistance and sensitivity of bacteria to antibiotics**

| code | Type of antibiotic | Resistance of isolates to the antibiotic % | Sensitive of isolates to the antibiotic % |
|---|---|---|---|
| **NF** | **Nafcillin** | **94.7%** | 5.3% |
| **AZM** | **Azithromycin** | **94.7%** | 5.3% |
| **CTX** | **Cefotaxime** | **89..4%** | 10.6% |
| **ATM** | **Aztreonam** | **73.6%** | 26.6% |
| **VA** | **Vancomycin** | **57.8%** | 42.2% |
| **GN** | **Gentamicin** | **47.3%** | 52.7% |
| **TE** | **Tetracycline** | **47.3%** | 52.7% |
| **CIP** | **Ciprofloxacin** | **42.1%** | 57.9% |
| **IMP** | **Imipenem** | **26.3%** | 73.7% |
| **MEM** | **Meropenem** | **20.5%** | 79.5 |

Baker's yeast collection and identification:

The diagnostic results showed that all isolates approved in the study, as shown in Table-3, are of the *S. cerevisiae* type.

**Table-3:** Number of types and origins of baker's yeast

| Sequence | Name | Origin | Code |
|---|---|---|---|
| 1 | Pakmaya | Turkey | Pa |
| 2 | Super maya | Iran | Su |
| 3 | Saf-instant | France | Sa |
| 4 | Eagle | Russia | Ea |
| 5 | Angel | china | An |

**Selecting the most efficient isolation baker's yeast:**

The Pa isolate was selected as the most efficient for producing bioemulsifier, with an emulsifying efficiency of E24=81.8%. Followed by Su and An with an efficiency of E24=77.2%, Then Saf and Na with an effectiveness of E24=75.0%. The difference in the ability of isolates to produce bioemulsion may be attributed to a difference in the quality

of the isolates. Growth conditions, including temperature, sugar, and oil concentration, play a role in production capacity. As well as the difference in gene expression that plays a role in the formation of components of the cell wall of yeast, thus affecting the production ability of the bioemulsion (Saleh et al., 2020).

**Measurement of the components of the bio-emulsion:**

The protein concentration of Pa yeast reached 80.82 mg/ml. Carbohydrate concentration: 780.11 µg/ml. With E24 emulsifying effectiveness = 81.8%. Therefore, the carbohydrate content of the bio-emulsion is many times greater than the protein content. It matches what was also found in previous studies by Saleh (2021) and Yan et al (2022).

**Investigate *C. myxa* extracts on some efficacious compounds:**

Both the water and alcoholic extracts contain flavonoids and glycosides, as well as phenols. The dominance of the alcoholic extract is that it contains alkaloids and saponins in concentrations more significant than their concentration in the water extract, As mentioned in Table 4. Al-Khafaji and his colleagues studied the components of the *C. myxa* plant and concluded that the plant is rich in highly effective compounds, including flavonoids, glycosides, and others. Despite the low concentration of alkaloids in the water extract, This is likely due to the low solubility of alkaloids in polar solvents such as water. The saponin reagent showed that the concentration of saponins in the water extract is low as a result of its high solubility, unlike the alcoholic extract, and this is consistent with the findings of Murad et al (2021).

**Table-4**: Qualitative detection of active chemical compounds

| compounds | Reagent used | Extracts | | Indication of reagent |
|---|---|---|---|---|
| | | WE | AE | |
| Phenols | Ferric chloride | +++ | +++ | Greenish brown precipitate |
| Flavonoids | NaoH detector | ++ | ++ | Yellow color appears |
| Alkaloids | Mayer's reagent | - | + | White precipitate |
| Glycosides | Keller Killiani Test | ++ | +++ | The appearance of a reddish-purple ring |
| Saponins | Foam | + | ++ | Thick foam that stays on for a while |

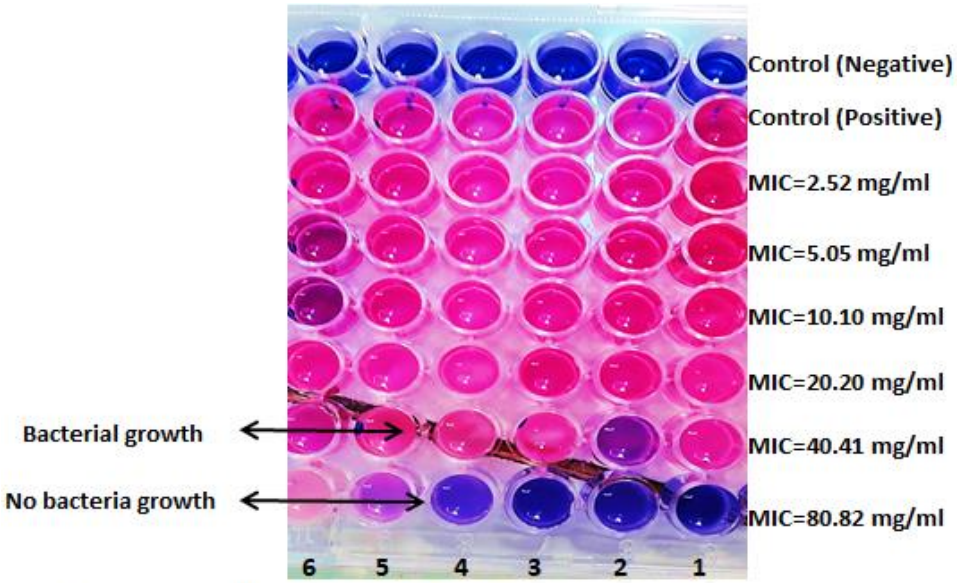
+ = positive detection. ++ = strong positive detection. +++ = Very strong positive detection. - = Negative detection.

**The Inhibitory activity of bioemulsifier against pathogenic bacteria**

The inhibitory activity test was conducted against six isolates of pathogenic bacteria, Which showed multiple resistance to antibiotics. The microtiter plates method was adopted, as in Figure 1. The MIC was determined using a Resazurin stain. The holes that retained their blue color did not have any bacterial growth in contrast to the holes that showed a pink color, indicating bacterial growth. Control positive was adopted without adding the bioemulsifier, so the holes appeared pink, And control negative was in blue due to the absence of bacterial culture.

The bioemulsion extracted from Pa yeast showed inhibitory activity with a minimum inhibitory concentration(MIC) of 80.82 mg/ml against all isolates of *Salmonella Typhi, Shigella sonnei, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae*, and *Escherichia coli.* These results are consistent with the findings of Naser (2015), Raham (2017), and Alizadeh-Sani et al (2018) that the bioemulsifier extracted from *S. cerevisiae* yeast has inhibitory activity against pathogenic intestinal bacteria. A study by Kazim (2019) found that the emulsion possesses antibacterial activity. A study by H.G.H. Rasheed and N. H. Haydar (2023) showed that the bioemulsion extracted from *S. cerevisiae* yeast has inhibitory activity against *P. aeruginosa* and *St. aureus* and the biofilm it forms, which is considered one of the virulence factors for bacteria.

One of the ways that the emulsion kills bacteria is by changing their permeability, which results in the release of ions and cell death. The bioemulsifier can interfere with the formation of bacterial biofilms and inhibit their formation (Minf and Ghribi, 2015).




1= *Salmonella Typhi*, 2= *Shigella sonnei*, = *Pseudomonas aeruginosa*,
4= *Staphylococcus aureus*, 5= *Klebsiella pneumoniae*, 6= *Escherichia coli*

**Figure-1**: Inhibitory effectiveness of bioemulsion against pathogenic bacteria

**The Inhibitory activity of Extracts *C. myxa* against pathogenic bacteria:**

The inhibitory activity of the water and alcoholic extracts against six bacterial isolates was tested, as shown in Figure 2. The water extract showed inhibitory activity against *S. Typhi, Shigella sonnei, P. aeruginosa, St. aureus*, and *K. pneumoniae* at the minimum inhibitory concentration (MIC) = 250mg/ml And at MIC=62.5mg/ml towards *E. coli*. The alcoholic extract showed inhibitory activity against *S. Typhi, Shigella sonnei*, and *K. pneumoniae* isolates. and *E. coli* at MIC=62.5mg/ml, While it showed inhibitory activity against *P. aeruginosa* and *St. aureus* isolates at MIC=125mg/ml.

The inhibitory capability of *C. myxa* extracts toward microorganisms is due to the potent compounds they contain. Alkaloids can cause damage to the permeability of the cell wall, resulting in an internal change in the chemical structure of proteins and, thus, the death of bacterial cells (Al-Maliki et al., 2021). The role of flavonoids in reducing the virulence of pathogenic bacteria by inhibiting the beta-lactamase enzyme and turning off the protein pump (Murad, 2021). The substance that has yet to receive attention from researchers despite its high ability to inhibit microorganisms is mucilage.

The high concentration of alkaloids in the alcoholic extract exceeds its concentration in the water extract. This difference is due to the solubility of the alkaloids between the extracts. Alkaloids have a higher solubility in less polar solvents, such as alcohol, unlike water, which is more polar. Thus, the inhibitory capability of microorganisms in the water extract decreased, in contrast to the alcoholic extract, which had a higher capacity (Al-Musawi et al., 2022).

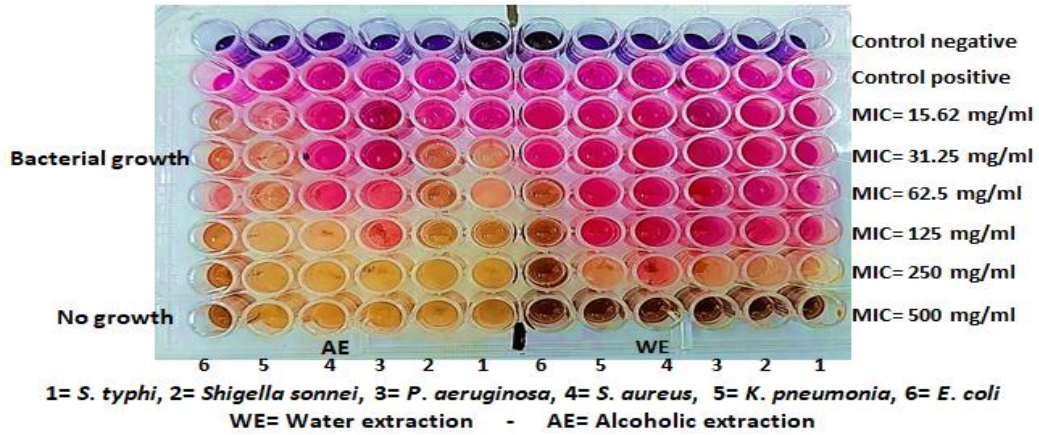



**Figure-2**: Inhibitory activity of water and alcoholic extracts of *C. myxa* fruits against pathogenic bacteria

**The synergistic effect of alcoholic extract and bioemulsifier against pathogenic bacteria:**

The synergistic relationship was conducted between the alcoholic extract of *C. myxa* fruit at half-diluted concentrations (500, 250, 125, 62.5, 31.25, 15.62, 7.81)mg/ml with the bioemulsifier extracted from *S. cerevisiae* yeast at half-diluted concentrations (80.82, 40.41, 20.20, 10.10, 5.05, 2.52, 1.26)mg/ml. The study showed, as shown in Table-5, a synergistic relationship exists between the extracts with a fractional inhibitory concentration (FIC) = 0.267 towards isolates of *S. typhi, Shigella sonnei, K. pneumonia*, and *E. coli*. Meanwhile, the FIC = 0.151 towards *P. aeruginosa* and *St. aureus* isolates. The work results have a synergistic effect, which means that the extracts' compounds interact with each other against pathogenic bacteria.

**Table-5:** The synergistic effect and (MIC) of the bioemulsion and the alcoholic extract with the (FIC) for both of them using the Boardchequer method and the type of relationship between them.

| isolate | MIC for Alcoholic extraction | MIC in combined for Alcoholic extraction | MIC for Mannoprotein | MIC in combined for Mannoprotein | FIC | FIC Index |
|---|---|---|---|---|---|---|
| ***S. typhi*** | 62.5 | 15.62 | 80.82 | 2.52 | 0.267 | Synergistic |
| ***Shigella sonnei*** | 62.5 | 15.62 | 80.82 | 2.52 | 0.267 | Synergistic |
| ***P. aeruginosa*** | 125 | 15.62 | 80.82 | 2.52 | 0.151 | Synergistic |
| ***St. aureus*** | 125 | 15.62 | 80.82 | 2.52 | 0.151 | Synergistic |
| ***K. pneumonia*** | 62.5 | 15.62 | 80.82 | 2.52 | 0.267 | Synergistic |
| ***E. coli*** | 62.5 | 15.62 | 80.82 | 2.52 | 0.267 | Synergistic |

## 4. Conclusion

The alcoholic extract of *C. myxa* fruits has a high inhibitory activity against pathogenic bacteria because it contains active compounds such as alkaloids; in addition to containing mucilage, The method of drying the fruits plays a role in the survival of this mucilage substance. Most importantly, the bioemulsifier extracted from *S. cerevisiae* has a synergistic effect with the alcoholic extract of *C. myxa* fruit, so it is recommended for use in the pharmaceutical and biotechnology industries.

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