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RESEARCH ARTICLE

The efficacy of newly formula of Alkanna tinctoria oil for treatment of five bacterial species

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Abstract

Background: According to World Health Organization (WHO), infectious diseases cause one of every six deaths worldwide. Bacterial infections are mostly treated with different types of antibiotics but development of bacterial resistance against these antibiotics makes treatment complicated. Some plants produce antimicrobial peptides as a part of their defense system, these naturally occurring peptides are seen as a promising alternative to regular antibiotics, because they are effective and save. The aim of this study is to evaluate the efficacy of newly discovered formula consists of Alkanna tinctoria, Myrrh , Custard, Lhaltih , Patience and olive oil in treatment of five bacterial species

Material and methods: The newly discovered formula consists of Alkanna tinctoria , Myrrh , Custard, Lhaltih , Patience and olive oil were tested on some common bacterial species including Staphylococcus aureus, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa and Escherichia coli by by using disk diffusion method

Results and conclusion: The study demonstrated that newly discovered formula consists of Alkanna tinctoria , Myrrh , Custard, Lhaltih , Patience and olive oil exhibit no effect against gram negative bacteria Proteus mirabilis, Pseudomonas aeruginosa and Escherichia but showed a very promising antimicrobial activity against S.aureus and E. faecalis species respectively and characterized by low-cost and more safe with no side effect.

INTRODUCTION

Review of literature

Infectious diseases cause one of every six deaths worldwide. Bacterial infections are mostly treated with different types of antibiotics depends on the type of bacteria that is causing the infection but after some time this bacteria gain resistant from the antibiotics that not be effected the bacteria and so the disease must be treated with another antibiotics types. (1)

Based on the mechanism of action, antibiotics are categorized into several classes. Antibiotics can be bactericidal or bacteriostatic through inhibiting the synthesis of cell wall, DNA, RNA, and protein, cell growth, and cell division (2).

Development of bacterial resistance against these antibiotics makes treatment complicated. Several reasons causing this problem including use of antibiotic in sub-therapeutic dose that favors bacterial resistance, immunosuppression due to malnutrition and suboptimal hygiene. The problem increased by using antibiotics in agriculture and treatment of animal disease so the discovery of new effective and save antibacterial material are necessary. (3)

Some plants produce antimicrobial peptides as a part of their defense system. These naturally occurring peptides are seen as a promising alternative to regular antibiotics, because they are effective and save. (4)
Roots of Alkanna tinctoria (AT) have been used for the treatment of wounds since ancient times and its medicinal property confirmed experimentally (5). The active components identified by Papageorgiou (6). The hexane extract of the root was divided into: waxes, fluorescent compounds, natural polymers, and pigments. (7)
The pigment containing fraction showed excellent healing effects, with the other fractions being completely inactive. Chemical analysis of the pigments identified the following esters of alkannin, dimethylacrylate , angelate, isovalerate and the novel b-acetoxyisovalerate (8) .
Aim of the work The aims of this work is to estimate the efficacy of newly discovered formula consists of Alkanna tinctoria, Myrrh , Custard, Lhaltih , Patience and olive oil in some common infected bacteria

Material and methods

Chemicals.
All compound were brought from the Herbs shops in Dawadme Governate

1. Myrrh (20 gram)
2. Custard (20 gram)
3. Lhaltih (20 gram)
4. Patience (20 gram)
5. Alkanna tinctoria (200 gram)
6. Olive oil (250 ml)

How to prepare the formula

1. Weight 200 grams of roots of Alkanna tinctoria .
2. Root was changed into a powder
3. Add 250 ml of olive oil on the roots .
4. Add 20 grams of the Myrrh, Custard, Lhaltih and Patience
5. Heat the mixture with stirring for 15 minutes (the mixture had red color)
6. Cool the mixture for one hour
7. filtered the mixture and put in a clean, dry bottle

Testing bacteria
The bacteria were obtained from the culture collections of the Department of Microbiology at The Dawadmi General hospital, Riyadh, Saudi Arabia . The organisms were as follows:

1. Staphylococcus aureus (Gram positive)
2. Enterococcus faecalis (Gram positive)
3. **Proteus mirabilis (**Gram Negative)
4. Pseudomonas aeruginosa (Gram Negative)
5. Escherichia coli (Gram Negative)

Antibacterial activity was done by the disk diffusion method (9), it was performed by using 10 ml Mueller Hinton Broth in an 24 h culture at 37°. Sterile saline solution was used to adjusted colonies at approximately 10^5 CFU/ml. In order to get a uniform microbial growth 500 microliters of the suspensions were spread over the plates containing Mueller Hinton agar using a sterile cotton swab on both control and test plates. The oil formula sterilized by filtration through a 0.45 µm membrane filter. Under aseptic conditions, oil formula diluted in pure olive oil and a 50 µL of different concentrations (1:1, 1:5, 1:10) of the (oil formula : pure olive oil are placed on the agar surface. We used 50 µL from standard concentration solution of Gentamicin , Ampicillin, Tetracycline and Azithromycin as reference positive control according to bacterial type.
All petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37°C for 24 hr.) After the incubation period, the zone of inhibition was measured with a caliper. Studies were performed in triplicate.
Statistical analysis.
The data were expressed as means ± standard errors (SEM). Differences between samples were estimated using a one-way analysis of variance (ANOVA) and a Duncan test was performed for multiple comparisons using the SPSS 12.0 for Windows. Results were considered as statistically significant at $P < 0.001$.

Results

The antibacterial activity of the new formula against tested bacterial species is summarized in table no 1,2,3,4 and 5. The results revealed that our new formula showed antibacterial activity with varying magnitudes on different species.

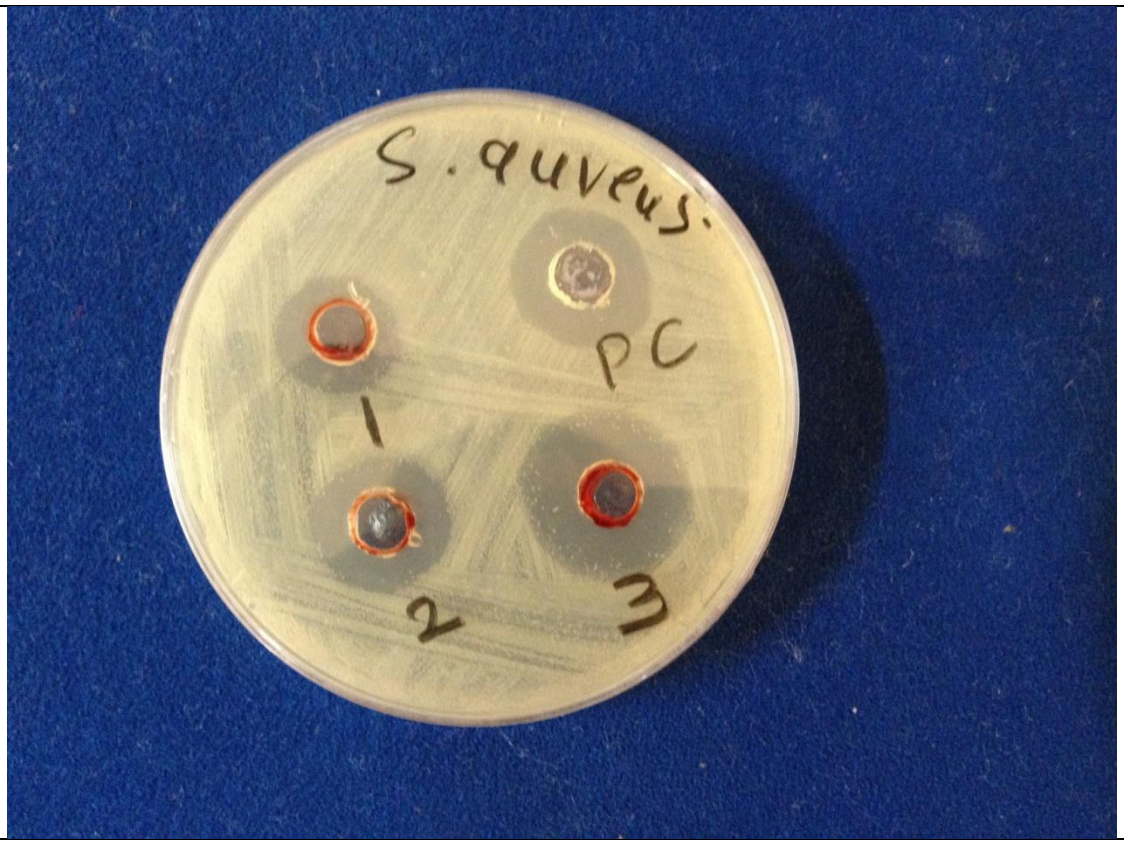


Fig (2) shows the effect of the formula on the S.aureus species

Pc is positive control (gentamicin)

1,2 and 3 is the formula diluted in different concentrations as (1:1, 1:5, 1:10) respectively

	Item	The mean size of Inhibition zone in mm	Percent (%)
1	**Positive control**	**5 ±0.2**	**100**
2	**Formula 1:10**	**4±0.1**	**80**
3	**Formula 1:5**	**5±0.2**	**100**
4	**Formula 1:1**	**8±0.2**	**160**

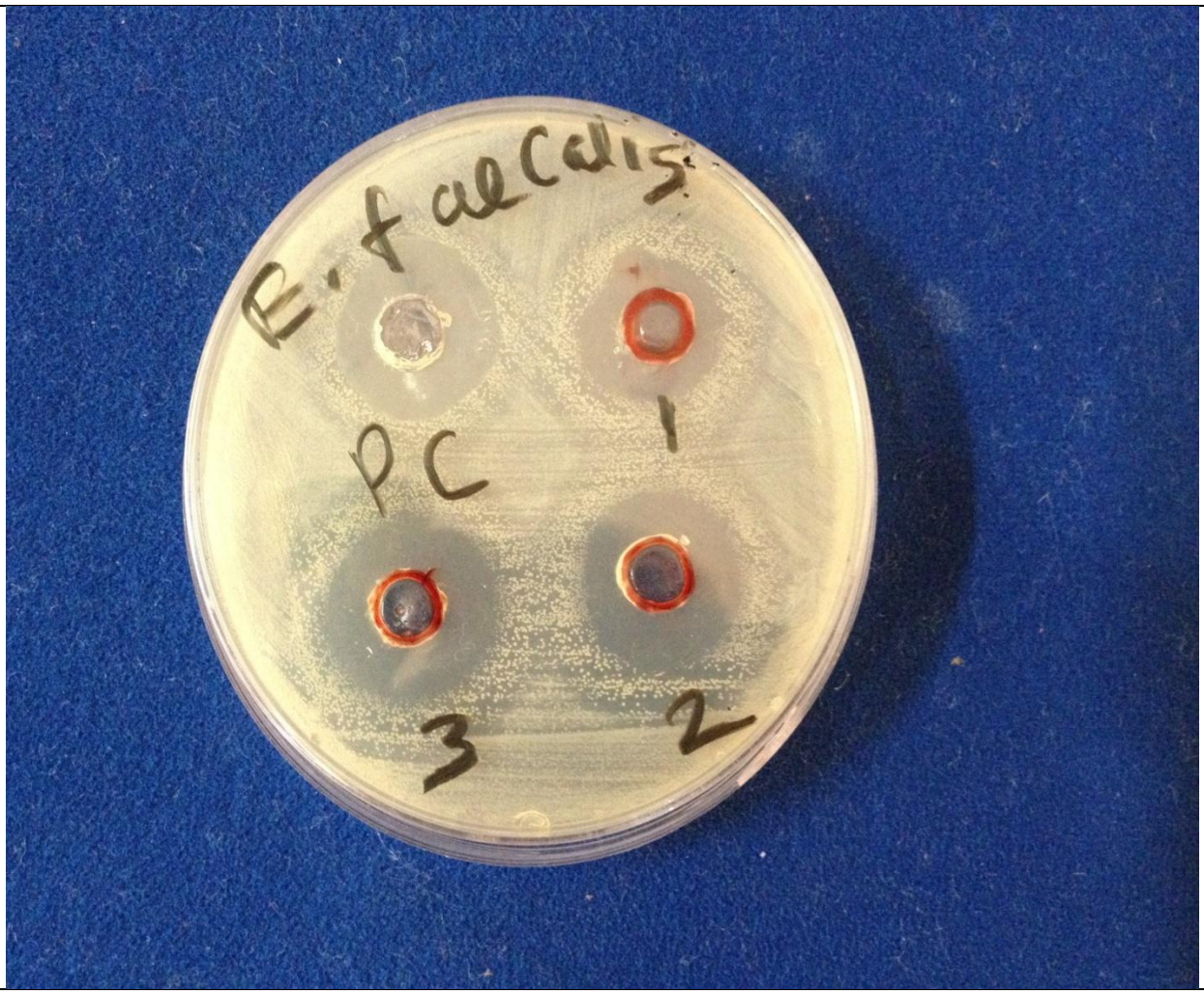


Fig (3) shows the effect of the formula on the Enterococcus faecalis species

Pc is positive control (ampicillin)

1,2 and 3 is the formula diluted in different concentrations as (1:10, 1:5, 1:1) respectively

	Item	The mean size of Inhibition zone in mm	Percent (%)
pc	**Positive control**	**5 ±0.1**	**100**
1	**Formula 1:10**	**4.5±0.3**	**90**
2	**Formula 1:5**	**5.5±0.2**	**110**
3	**Formula 1:1**	**7±0.2**	**140**

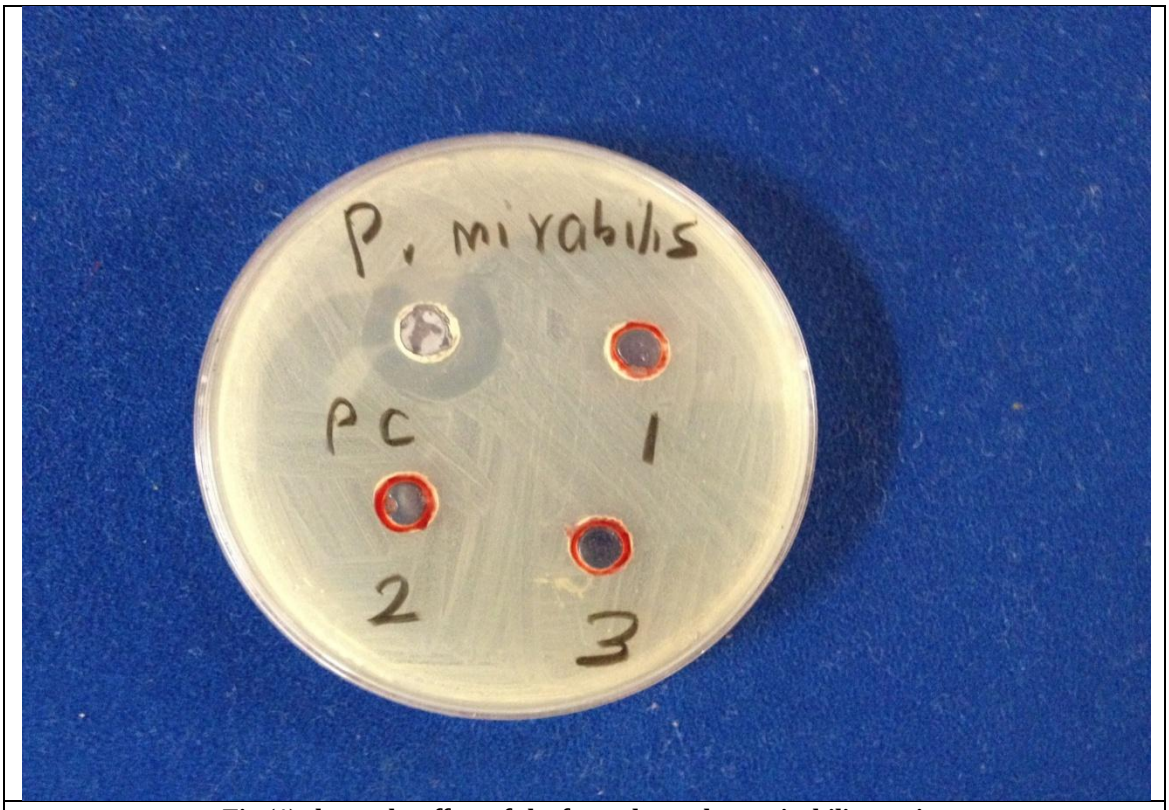


Fig (1) shows the effect of the formula on the p.mirabilis species
Pc is positive control (tetracycline)
1,2 and 3 is the formula diluted in different concentrations as (1:1, 1:5, 1:10) respectively

	Item	The mean of Inhibition zone size in mm	Percent (%)
pc	**Positive control**	**5 ±1**	**100**
1	**Formula 1:10**	**0**	**0**
2	**Formula 1:5**	**0**	**0**
3	**Formula 1:1**	**0**	**0**

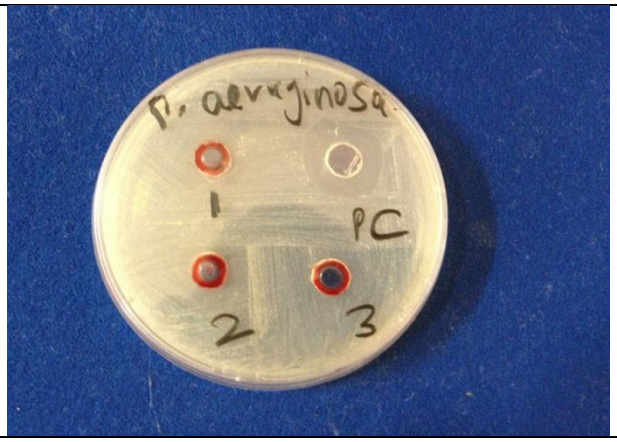


Fig (4) shows the effect of the formula on Pseudomonas aeruginosa species
Pc is positive control (gentamicin)
1,2 and 3 is the formula diluted in different concentrations as (1:10, 1:5, 1:1) respectively

	Item	The mean size of Inhibition zone in mm	Percent (%)
pc	Positive control	5 ±1	100
1	Formula 1:10	0	0
2	Formula 1:5	0	0
3	Formula 1:1	0	0

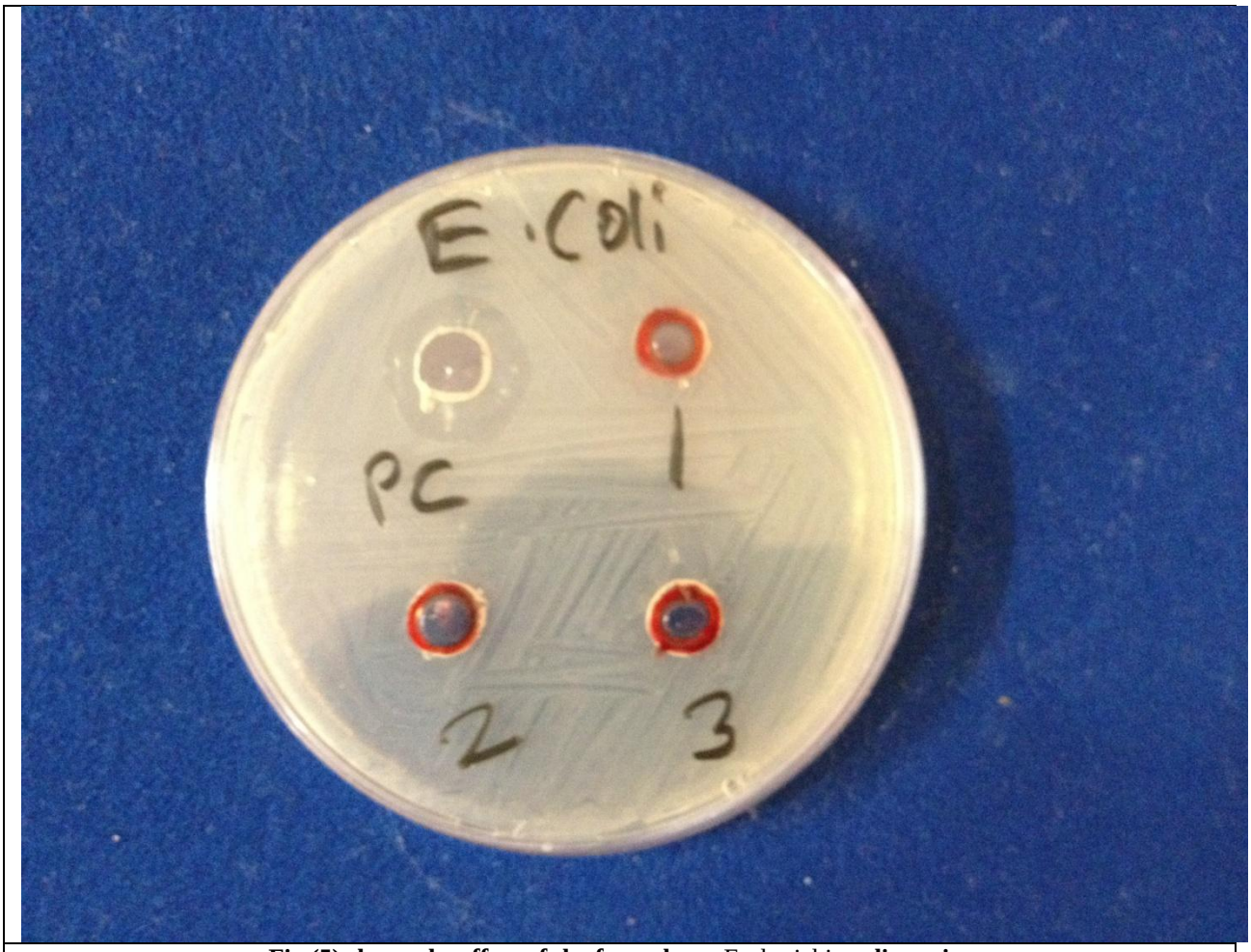


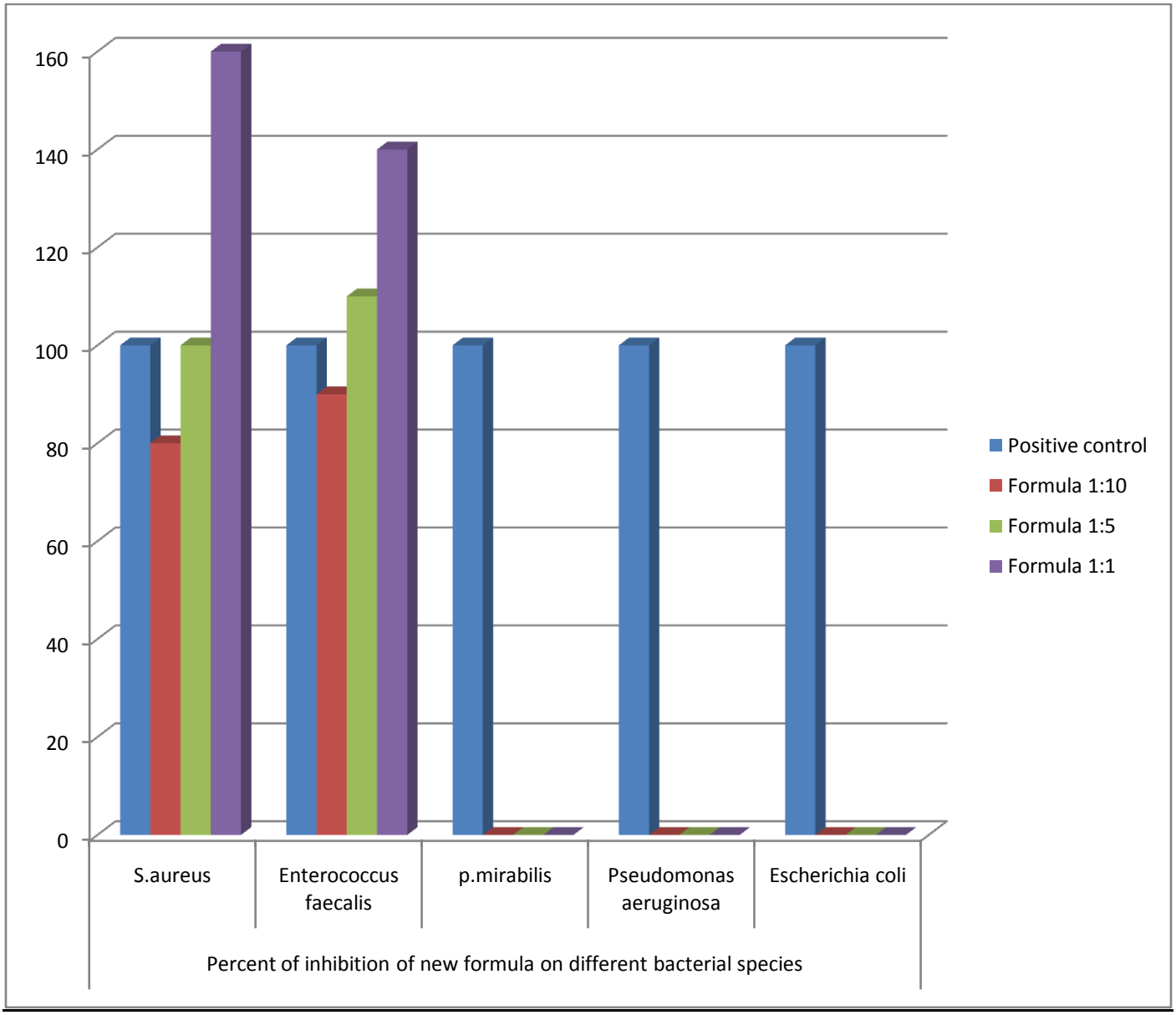
Fig (5) shows the effect of the formula on Escherichia **coli species**
Pc is positive control (azithromycin).
1,2 and 3 is the formula diluted in different concentrations as (1:10, 1:5, 1:1) respectively

	Item	The mean size of Inhibition zone in mm	Percent (%)
pc	Positive control	5 ±0.1	100
1	Formula 1:10	0	0
2	Formula 1:5	0	0
3	Formula 1:1	0	0

Item	Percent of inhibition of new formula on different bacterial species

	S.aureus	Enterococcus faecalis	p.mirabilis	Pseudomonas aeruginosa	Escherichia coli
Positive control	**100**	**100**	**100**	**100**	**100**
Formula 1:10	**80**	**90**	**0**	**0**	**0**
Formula 1:5	**100**	**110**	**0**	**0**	**0**
Formula 1:1	**160**	**140**	**0**	**0**	**0**

Summary



Discussion

In 2010, Center of Disease Control reported that bacterial infection results approximately 30,000 deaths each year in the United States. It is estimated that annual cost to treat infections of six major bacteria was over $1.87 billion.(10) In this study we try to test the effect of new formula consists mainly from Alkanna tinctoria root extract which have high levels of alkannine. The active ingredients alkannine used as food coloring and they reported to posses anti inflammatory activity. (11).

The results demonstrated that newly discovered formula consists of Alkanna tinctoria , Myrrh , Custard, Lhaltih , Patience and Olive oil exhibit a very promising antimicrobial activity .
From the zone of inhibition we observe that the formula was sensitive to gram positive bacteria and not sensitive against gram negative bacteria in the tested organisms. many previous studies indicated that gram-negative bacteria intrinsically exhibit higher drug tolerance than gram positive microbes (12)
. The formula showed maximum activity against bacterial S.aureus and Enterococcus faecalis species respectively .
The previous studies showed that natural occurring pigments of Alkanna tinctoria play an important role in the biochemistry of living cells and exert several biological activity and this may due to their ability to act as potent inhibitors oxidative phosphorylation uncopling, intercalating the DNA, reactive oxygen molecules (**6**)
If we compared the cost of new formula and its promising effects with the average for using other treatments we will find it is low-cost and more safe with no side effects than alternative preparation for the treatment of S.aureus and Enterococcus faecalis species respectively .

CONCLUSION:

The study demonstrated that newly discovered formula consists of Alkanna tinctoria , Myrrh , Custard, Lhaltih , Patience and olive oil exhibit a very promising antimicrobial activity and characterized by low-cost and more safe with no side .

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