

THE EFFECT OF MEDICINAL PLANTS AND THE GENTAMICIN AGAINST SELECTED BACTERIA: IN VITRO STUDY

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Abstract

Antimicrobial-resistant bacteria are becoming more serious danger to worldwide public health. As a result, the efficacy of traditional antimicrobials is fast falling, posing a threat to healthcare providers' capacity to treat common infection. Bacterial infective diseases are an important reason of sickness and death globally. Several medicine resistances in human pathogenic microorganisms had develop because of indiscriminating custom of commercial anti-microbial medicines.

The main objective of the present study is, to evaluate the ability of the plants extract, to inhibit the growth of pathogenic bacteria with and without antibiotics and non-antibiotics drugs. Also, to enhance the activity of antibiotics or non-antibiotics drugs against selective pathogenic bacteria, to investigate antibacterial effects of thyme crude extract (Tm), ginger crud extract (Gi), garlic crud extract (Ga) and acacia crud extract (AC) against Escherichia coli (E.coli) and Staphylococcus aureus (S.aureus) and to investigate the ability of increasing the potency of Gentamycin antibiotic against E. coli and S. aureus by mixed with thyme crude extract (Tm), ginger crud extract (Gi), garlic crud extract (Ga) and acacia crud extract (AC).

Material and methods, in this study used gentamicin mixed with for types of crud extracts garlic, ginger, acacia, and thyme against two types of bacteria E. coli and S. aureus. Used disc diffusion method. Results found that three types had increasing the effect of gentamicin

against *S. aureus* and had not affect against *E. coli*. Also, increased the potency of gentamicin against *S. aureus* when combined with garlic, thyme and acacia crud extracts.

Keywords: (*garlic, ginger, acacia, thyme, and Gentamicin*).

INTRODUCTION

Infectious diseases caused by bacteria are a major cause of illness and death around the world. Therefore, one of the biggest successes of modern medicine is the development of antimicrobials to treat infectious infections. Natural goods are a major source of medications and medication development. More than 60% of residents in the world are trusting on natural plant product particularly in unindustrialized countries, people in these countries used plant natural products indirectly to maintain a healthiness [1].

But, in industrial countries plant extracts are rarely used as antimicrobials or as a systemic antibiotic due to their low level of activity against gram-negative bacteria by measuring their applicability and benefits by expending modern scientific analysis methods [2]. In the ancient times, garlic (*Allium sativum*) and onion (*Allium cepa*) had an imperative dietary source and involved for medicinal determinations. The garlic is representative genus of the Liliaceae family, which contains 450 species [3].

Many studies on healing properties of garlic as antibacterial, antiviral, antifungal, antiprotozoal, anticancer, antioxidant, immuno-modulatory, and anti-inflammatory were done [4]. Also, Garlic not used as food only, but also used as medicine for many sicknesses [5]. In addition, the genus *Zingiber* (*Ginger*), fitting to Zingiberaceae's family, includes in 85 species of herbs of Africa, Asia, South America and Central America [6].

Ginger (*Zingiberofficinale* Roscoe) is a rhizomatous perennial herb, attainment up to 90 cm in the long. The rhizomes of ginger are pale yellowish color, aromatic, lobed, bearing simple alternate distichous thin leaves. *Ginger* grows numerous of lateral shoots in bunches, which dried while it matures. Flowers are rare, rather small, calyx superior, gamosepalous, three toothed, on one side they are splitting, corollas are greenish segments [7]. *Ginger* rhizomes are eminent herbal consumed as a spice which uses in food and traditional medicine. Several studies showed their antibacterial activities and showed

different effects [1]. But, *Acacia arabica* L belongs to Abaceae's family; *Acacia*'s seeds used for varieties products and foods and used as an active drug for diarrhea [8]. The evergreen trees are usually creating at waterless areas [9]. *A. arabica* is normally create in woof of the tree which fits to family Leguminosae. Management of *A. arabica*'s seed ground was produce insulin from pancreatic beta cells.

However, *Thymus vulgaris* L (*T. vulgaris*), fits to the family Lamiaceae, and have several uses. It increases a distinctive aromatic flavoring to pulps, stuffing, meats and poultry; it possesses anti-oxidative properties, antiseptic, antispasmodic, carminative and expectorant [10]. While, the inhibitory activity of diverse antibiotics application on different pathogenic organisms were done. But in modern years, several medicine resistances in human pathogenic microorganisms had develop because of random custom of commercial anti-microbial medicines, generally used in the treatment of infectious diseases. The increase of antibiotic resistance including the specific nature to linked of bacteria. This condition has forced scientists to search for effects and can be natural sources that act new anti-infectious agents [11].

Several studies have been done in for control the antimicrobial action of many medicinal plant extracts. Shakurfow et al (2015) had tested the actions of garlic water extracts against *Listeria monocytogenes* [3]. Also, Wei et al., 2005 and Arshad and Shadab, 2017, were they studied ginger extracts as antibacterial strength. Moreover, Gupta and Ravishankar, (2005) tested the antimicrobial effects of ginger extract in peptone water buffer against *E. coli* O157:H7.

In addition, the antibacterial activity of the *T. vulgaris* and *Eucalyptus camaldulensis* was studied against *L. monocytogenes* [12]. ginger water extracts have diverse degrees of antibacterial activity water ginger extracts also evaluate the synergistic effect of this extract with amoxicillin and gentamicin antibiotics. A new antibacterial agent from natural sources was required by the rise of antibiotic resistant bacteria because of the random use of antibiotics [13].

Also, ginger extracts have different degrees of antibacterial effectiveness [13, 14], where the essential oil of ginger was stronger than the oleoresin against *E. coli* [15]. Whereas, the antimicrobial effects of ginger extract in peptone water buffer

against *E. coli* O157:H7 was strong [16]. Where, in a scientific experimental with Gum (a commercially available gel containing *A. arabica* showed significant medical advanced in gingival and plaque

index scores as compared to a control gel. Gum gel was not associated with any discoloration of teeth or unpleasant taste [9].

THE MAIN OBJECTIVE OF THE PRESENT STUDY ARE

1-To evaluate the ability of the plants, extract to inhibit the growth of pathogenic bacteria with and without antibiotics and non-antibiotics drugs and to determine their ability.

2-To enhance the activity of antibiotics or non-antibiotics drugs.

3-To investigate antibacterial effects of thyme crude extract (Tm), ginger crud

extract (Gi), garlic crud extract (Ga) and acacia crud extract (AC) against *E.coli* and *S. aureus*.

4-To investigate the ability of increasing the potency of Gentamycin antibiotic against *E. coli* and *S. aureus* by combined with thyme crude extract (Tm), ginger crud extract (Gi), garlic crud extract (Ga) and acacia crud extract (AC).

MATERIALS AND METHODS

Materials

Plant material:

Garlic bubbles, Fig (1)



Figure 1: Garlic plant bulb and scrunch

Ginger raizomes, Fig (2)



Figure 2: Zingiber rhizomes and powder

Acacia lives, Fig (3)



Figure 3: A. arabica L powder

Thyme lives, Fig(4)



Figure 4: T. vulgaris dry leaves and powder

Microorganisms

Pathogenic strains of *S. aureus* Fig (5) and *E. coli* Fig (6) were obtained from medical laboratory department/Al-Khomes central

hospital. All strains were cultured in Nutrient agar at 37°C for 24 hrs and then stored at 4 °C for further experiments.



Figure 5: S. aureus on blood agar



Figure 6: E. coli on blood agar

Methods

Preparation of Plant Extracts

The method of preparation of crude plant extracts of Mohamedin, et al. (2018) was adopted. The powder of ginger dry rhizome, garlic dry bulbs, Thyme dry leaves were purchased from the local

market of Libya and dried at room temperature. thyme crude extract (Tm), ginger crude extract (Gi), garlic crude extract (Ga) and acacia crude extract (Ac). The test microorganisms were spread on

nutrient agar medium by with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5mm in diameter) inoculated with the test microorganisms and then impregnating with 10 µl of plant crude

Antibacterial Bioassay of Ginger

Extracts

To evaluate the antibacterial activity of the Ga, Ac, Gi, and Tm crude extracts by the disc agar diffusion method was used Mohamedin et al., (2018) Ten ul of each

Synergistic Effect of The Extracts with antibiotics, If Any

The method of synergistic effect of the extracts with antibiotics of Mohamedin, et al. (2018) was done. Single impact of Ga, Ac, Gi, and Tm crude extracts with Gentamicin (CM) as well as combinations of CM+Ga, CM+Ac, CM+Gi, and CM+Tm were performed commercially antibacterial AX discs and GM discs containing 10 ug were saturated with 10 ul each extract under aseptic conditions and then were applied on the

STATISTICAL

Used percentage for measured the increased of gentamicin potency.

RESULTS AND DISCUSSION

extract. The plates were subsequently incubated at 37°C for 24 hours. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm

crude extract as well distilled water against pathogenic bacteria mentioned before. The diameter of inhibition zones (mm) were measured after 24 h incubation at 37°C. Antimicrobial activity was recorded when the zone of inhibition is greater than 5 mm.

surface Nutrient agar media freshly inoculated by the tested bacteria. The plates were incubated at 4°C for 20 min and then were transferred to 37°C for 24 hrs after the incubation period the diameters of the inhibition zones formed on the media were measured in mm and then compared with each other.

The inhibition zone of crud plant extracts alone and combination between crude plant extracts and gentamicin against *E. coli* and *S. aureus* in Figs. (7), (8), (9), (10) and (11) were measured with ruler and then

recorded in tables (1) ,(2) ,(3) and (4) separately



Figure 7: garlic crud extract (Ga), gentamicin (CM), distillate water (DW) and combination between garlic and gentamicin (CM+Ga) against *S. aureus* (st).



Figure 8: Ginger crud extract (Gi), Gentamicin (CM), distillate water (DW) and combination between Ginger and gentamicin (CM+Gi) against *E. coli* (EC).

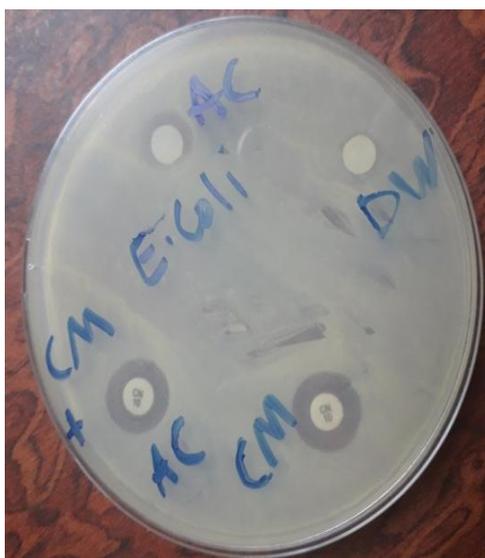


Figure 9: Acacia crud extract (AC), Gentamicin (CM), distillate water (DW) and combination between Ginger and gentamicin (CM+AC) against E. coli (EC).

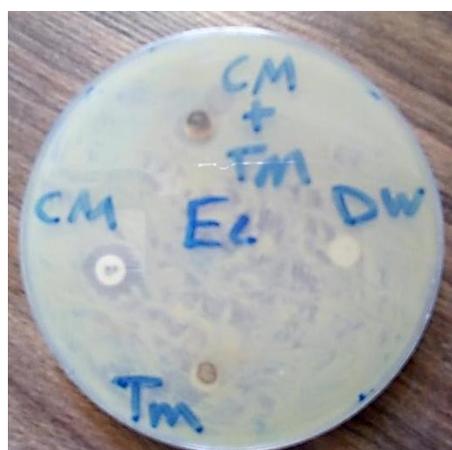


Figure 10: Thyme crud extract (Tm), Gentamicin (CM), distillate water (DW) and combination between Ginger and gentamicin (CM+Tm) against E. coli (EC).



Figure 11: Garlic crud extract (Ga), gentamicin (CM), distillate water (DW) and combination between ginger and gentamicin (CM+Ga) against *E. coli* (EC).

Table 1. Inhibition zone mm of combination between Acacia crud extract and gentamicin against tested bacteria

Materials	Inhibition zone mm			The distinction
	Single		Combination	
	AC	CM	AC+CM	
<i>E. coli</i>	07±2	16±0	16±0	00.0%
<i>S. aurous</i>	08±1	09±0	10±0	↑10.0%

AC= Acacia crud extract, CM=Gentamicin

Table 2. Inhibition zone mm of combination between Thyme crud extract and gentamicin against tested bacteria

	Inhibition zone mm			The distinction
Materials	Single		Combination	
Bacteria	Tm	CM	Tm+CM	
<i>E. coli</i>	14±2	14±0	13±1	↓07.2%
<i>S. aurous</i>	07±0	07±0	10±1	↑30.0%

Tm= Thyme crud extract, CM=gentamicin

Table 3. Inhibition zone mm of combination between Ginger crud extract and gentamicin against tested bacteria

	Inhibition zone mm			The distinction
Materials	Single		Combination	
Bacteria	Gi	CM	Gi+CM	
<i>E. coli</i>	08±0	13±2	12±1	↓8.0%
<i>S. aurous</i>	08±1	13±2	13±2	0.0%

Gi= ginger crud extract, CM= Gentamycin

Table 4. Inhibition zone mm of combination between garlic crud extract and gentamicin against tested bacteria

	Inhibition zone mm			The distinction
Materials	Single		Combination	
Bacteria	Ga	CM	Ga+CM	
<i>E. coli</i>	08±1	13±0	13±1	00.0%
<i>S. aurous</i>	07±1	26±2	31±2	↑16.1%

Ga= garlic crud extract, CM= gentamycin

The results in Table 1. showed inhibition zone by mm of combination between Acacia crud extract and gentamicin against tested bacteria against *E. coli* and *S. aureus* which were high against *S. aureus* 10.0%. Also, The results in Table 2. showed Inhibition zone by mm of combination between Thyme crud extract and gentamicin against tested bacteria against *E. coli* and *S. aureus* which were high effect against *S. aureus* 30.0%.

Similarly, The results in Table 4. showed Inhibition zone by mm of combination between garlic crud extract and gentamicin against tested bacteria against *E. coli* and *S. aureus* which were high effect against *S. aureus* 16.1%. But. The results in Table 3. showed Inhibition zone mm of combination between Ginger crud extract and gentamicin against tested bacteria against *E. coli* and *S. aureus* which have not against both types of bacteria. Shakurfow et al 2015, agree with this study in the effect of garlic against *S. aureus* but not agree with garlic crude extract against *E. coli* that is might be the researcher never sterilized the crud extract. But, Mohamedin et al 2018, whom tested the effect of ginger extracts against *S. aureus* and *E. coli* in contrast of this study, he had found high effect against these

bacteria [17], that is might be due to they used methanol for extraction. But, Lawrence et al 2015 decide with us in *S. aureus* despite they used other solvents. But, disagree with us in *E. coli* [18]. Also, this study agree with Nakamoto et al (2020) garlic-derived hydrophobic compounds may be used to enhance the effects of existing drugs and treatment infections because its preparations have broad-spectrum antimicrobial [5]. Although, Karuppiah and Rajaram, (2012) reported both the garlic and ginger extracts in zone inhibition diverse between 7 mm and 19 mm [19]. The garlic cloves ethanolic extract showed highest diameter of zone inhibition about 19.45 mm against *P. aeruginosa* followed by *E. coli* 18.50 mm and *Bacillus sp.* 16.5 mm. The garlic cloves ethanol extract showed almost similar zone inhibition = 13.50 mm in diameter against *Proteus sp.*, *S. aureus* and *Enterobacter sp.*

Also, Park et al., (2008) tested the ginger rhizomes ethanol extracts and demonstrated antibacterial activity against five clinical isolated with zone of growth inhibition ranging from 4 mm to 16 mm [20]. The maximum zone of inhibition was showed against *Bacillus sp.* 16.55 mm followed by *E. coli* 15.50 mm and *P. aeruginosa* 14.45 mm. The minimum

diameter of zone inhibition was recorded against *Klebsiella* sp. 5 mm and

Enterobacter sp. 4 mm.

CONCLUSION

The study concluded that Garlic, thyme and acacia crud extracts have potency against both types of bacteria against *E. coli* and *S. aureus*.

Gentamicin's potency did not increased against *E. coli* when gentamicin combine with garlic, thyme and acacia crud extracts.

Gentamicin's potency increased against *S. aureus* when gentamicin combine with garlic, thyme and acacia crud extracts.

RECOMMENDATION

From the conclusion of this study, the study recommended with must be combine between gentamicin and one of garlic, thyme and acacia crud extracts against *S. aureus* for increased the potency of the antibiotic gentamicin.

More studies must be done for increased the potency of gentamicin against pathogenic bacteria.

ABBREVIATIONS

Tm	Thyme Crude Extract
Gi	Ginger Crud Extract
Ga	Garlic Crud Extract
Ac	Acacia crud extract
CM	Gentamycin
DW	distillate water

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