https://doi.org/10.54361/ljmr15.1.12

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

Fathi Abdallah Shakurfow[1]*, Ali Salem Faraj Edalim[2],OsamaMilad Elgutt[3], Abdelkrem Mohamed Bashir Abdalla[4] and Abdurraouf Zaet[5]

[1,2,3] Medical Laboratory Department, Faculty of Medical Technology, El-Mergib Uni. Libya

[4] Medical Laboratory Department, Faculty of Medical Technology, Zawia Uni. Libya

[5]Dental Technology Department, Faculty of Medical Technology, Zawia Uni. Libya

*Corresponding Author: fathishakurfow@gmail.com

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 undiscriminating 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 bubles, 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^{o}Cfor 24 hrs and then stored at 4 ^{o}Cfor 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 crud 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 crud extract (Gi), garlic crud extract (Ga) and acacia crud 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 extract. The plates were subsequently incubated at 37°Cfor 24 hours. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm

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 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°CAntimicrobial activity was recorded when the zone of inhibition is greater than 5 mm.

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+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 surface Nutrient agar media freshly inoculated by the tested bacteria The plates were incubated at 4°Cfor 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.

STATISTICAL

Used percentage for measured the increased of gentamicin potency**.**

RESULTS AND DISCUSSION

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) separatly

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).

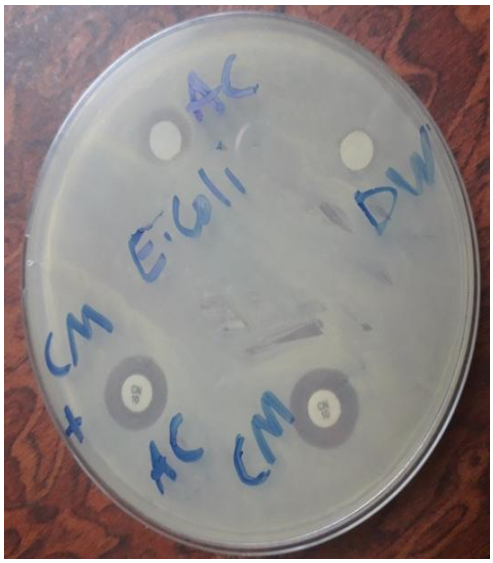

Figure 9: Acacia crud extract (AC), Gentamicin (CM), distillate water (DW) and combination between Ginger and gentamicin (CM+AC) against E. coli (EC).

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

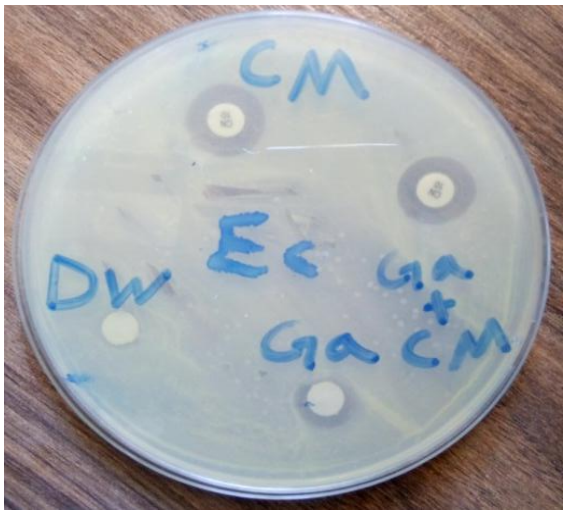

Figure 11: Garlic crud extract (Ga), gentamicin (CM), distillate water (DW) and combination between ginger and gentamicin (CM+Ga) against E. coli (EC).

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

	Inhibition zone mm			
Materials	Single		Combination	The distinction
Bacteria	AC	CM	AC+CM	
E. coli	07±2	16±0	16±0	00.0%
S. aurous	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			
Materials	Single		Combination	The distinction
Bacteria	Tm	CM	Tm+CM	
E. coli	14±2	14±0	13±1	↓07.2%
S. aurous	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	
E. coli	08±0	13±2	12±1	↓8.0%
S. aurous	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			
Materials	Single		Combination	The distinction
Bacteria	Ga	CM	Ga+CM	
E. coli	08±1	13±0	13±1	00.0%
S. aurous	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. aurous 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. aurous which were high effect against S. aurous 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 fond 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 increased against S. aureus when gentamicin combine with garlic, thyme and acacia crud extracts.

Gentamicin's potency did not increased against E. coli 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

ACKNOWLEDGMENT

The author would like to thank medical laboratory technics RawidaKhamis Saeed, FatmaMabrokAlgadafy, RimanFathiAlsharif and Najah Ali Ben-Ali for helpful.

REFERENCES

[1] Cragg, G. M., and Newman, D. J. (2013); Natural products: A continuing source of novel drug leads. BiochBiophysActa 1830(6):3670-95.

[2] Abdel Rahman, S., Abd-Ellatif, S., Deraz, S. and Khalil, A. (2011); Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. African Journal of Biotechnology, 10(52):10733-43.

[3] Shakurfow, F. A. A., Buazzi, M. M., and Gamal, M. A., Assessment of antimicrobial activity of onion (*Allium cepa*) and garlic (*Allium sativum*) extracts on *Listeria monocytogenes*; in vitro study. Lebda Medical Journal. 1(1):1-5.

[4] Harris, J. C., Plummer, S., Turner, M. P., Lloyd, D. (2000); The Microaerophilic Flagellate Giardia Intestinalis: *Allium sativum* (Garlic) is an Effective Antigiardial. Microbiology journal. 146 (12):3119-27.

[5] Nakamoto, M., Kunimura, K., Suzuki, J., and Kodera, Y. (2020); Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinyldithiin, ajoene and diallylpolysulfides (Review). Spandidos publications. Experimental and therapeutic medicine. 19(2):1550-1553

[6] Sabulal, B., Dan, M., Kurup, R., Pradeep, N. S., Valsamma, R. K., and George, V. (2006); Caryophyllene-Rich Rhizome Oil of *Zingibernimmonii*from South India: Chemical Characterization and Antimicrobial Activity. Phytochem 67: 2469-2473.

[7] Kawai T., (1994); Anti-emetic principles of *Magnolia obovata*Bark and *Zingiberofficinale*Rhizome. Planta Med. 60(1):17-20.

[8] Spicer, N., Barnes R., and Timberlake, J., (2007); *Acacia* handbook. DFID (2010). Short-term clinical effects of Forestry Research Programme, U.K.

[9] Pradeep, A. R., Happy, D. and Garg, G. (2010); Short-Term Clinical Effects of Commercially Available Gel Containing *Acacia Arabica*: a randomized controlled clinical trial. Australian Dental Journal, 55:65-69.

[10] Omidbaigi, R. and Nejad, R. A.. (2000); The Influence Of Nitrogen Fertilizer and Harvested Time on The Productivity of *T. vulgaris*. Inter. J. Horti. Sci., 6(3): 43-46.

[11] Jouda, M. M., Elbashiti, T., Masad, A., Albayoumi, M., (2015); The Antibacterial Effect of Some Medicinal Plant Extracts and Their Synergistic Effect With Antibiotics. *Research Article* 5(2):23-33.

[12] Al-Muhna, B. M., Study of the Inhibitory Effect of Thymus vulgaris and Eucalyptus camaldulensis on In vitro Growth of *Listeria monocytogenes*. (2010); Kufa Journal For Veterinary Medical Sciences 1(1).

[13] Arshad, M. and Shadab, M., (2017); *Zingiberofficinale* Extract: Antimicrobial Properties Phytochemical Screening, Drug Likeness and Physicochemical Studies. Ejpmr. 4(3):364-368.

[14] Wei, Q. Y., Ma, J. P., Cai, Y. J., Yang, L., and Liu Z. L., (2005); Cytotoxic and Apoptotic Activities of Diarylheptanoids and Gingerol-Related Compounds from The Rhizome of Chinese Ginger. Journal of ethnopharmacology. 102(2):177-184.

[15] Bellik, Y., (2014), Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of Zingiberofficinale Roscoe. Asian Pacific Journal of Tropical Disease. 4(1):40-44

[16] Gupta, S., and Ravishankar, S. (2005); A Comparison of the Antimicrobial Activity of Garlic, Ginger, Carrot, And Turmeric Pastes Against *E. coli* O157:H7 in Laboratory Buffer And Ground Beef. Foodborne Pathog Dis. 2(4):330-40.

[17] Karuppiah P. and Rajaram S. (2012) Antibacterial Effect of *Allium sativum* cloves and *Zingiberofficinale* Rhizome Against Multiple-Drug Resistance Clinical Pathogens. Asian J Biomedicine.; 2(8):597-601.

[18] Mohamedin, A., Elsayed A., and Shakurfow, F. A.Molecular Effects and Antibacterial Activities of Ginger Extracts Against Some Drug Resistant Pathogenic Bacteria (2018); Egypt. J. Bot., 58(1):133 -143.

[19] Lawrence R., Jeyakumar E. and Gupta A. (2015); Antibacterial Activity of *Acacia arabica* (Bark) Extract Against Selected Multi Drug Resistant Pathogenic Bacteria Int. J. Curr. Microbiol. App. Sci. Special Issue-1: 213-222.

[20] Park, M., Bae, J., Lee, D. S., (2008); Antibacterial Activity of [10]-Gingerol and [12]-Gingerol Isolated from Ginger Rhizome Against Periodontal Bacteria. *Phytotherapy*research : PTR 22(11):1446-1449.