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COMBINED ACTION OF SOME ESSENTIAL OILS AND ANTIBIOTICS ON BACTERIAL GIT-PATHOGENS

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Abstract

Susceptibility of some gastrointestinal tract (GIT) bacterial-pathogens to combinations of MRSA-growth inhibiting antibiotics (MGIA), MRSA growth non-inhibiting antibiotics (MGNIA) and some plant extracts, separately or in groups were investigated. The results revealed that combinations from MGNIA (Gentamycin with Co trimoxazole), MGIA (Vancomycin with Ciprofloxacin) and MGNIA with MGIA (Gentamycin with Imipenem) were determinative for the growth of most tested GIT-pathogens. On the other hand, aerial shoots of *Artimesia monosperma* L (Am), *Ocimum basilicum* L (Ob), *Origanum majorana* L (Om), *Salvia officinalis* L (So) and *Pelargonium graveolens* (Pg) were applied collectively in a mixture (PM) and yield the most lethal effect on the pathogens (13.2 IZD). Further total combination mixture (TM) of *Cannabis sativa* and *Foeniculum vulgare* seed mixture (SM) with PM increased the determinative impact on pathogens growth and produced more susceptibility (17.3mm IZD). GC/MC analyses have shown that the major essential oils content of TM were α-pinene, β-pinene, stragol, γ-terpinene, γ-terpinolene, caryophyllene, thugen, geranyl linolool, limonene and eugenol. Moreover, combination of TM with Gentamicin and Impenem (TMGI) had a further synergistic effect (25 mm IZD). The mechanism of action for TMGI mixture ingredient may be resulted from the disruption of bacterial cell membrane, blocking of protein synthesis and out diffusion of cellular components.

Keywords: GIT, bacterial-pathogens, MRSA, aerial shoots of plants.

1. Introduction

Enterobacteriaceae as a large heterogeneous group of gram-negative rods, comprise more than 20 species that have been identified to be natural inhabitants in the gastrointestinal tract (GIT) of humans and animals. However, clinically significant *Enterobacteriaceae* genera include *Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella*, *Serratia, Citrobacter, Proteus*, and others [1]. *Salmonella* and *Shigella* species are regularly pathogenic for humans and cause enterocolitis, while *E. coli* is part of the normal flora and incidentally cause a wide variety of infections. *Klebsiella pneumonia, Enterobacter cloacae* and *Serratia marcescens* are frequently found in the large intestine and they are often involved in human infections [2]. Multiresistance, not only to beta lactams, but also to quinolones, trimethoprim, tetracycline, and most aminoglycosides complicating therapy and causing serious problems in both community and hospital settings [3]. On the other hand, [4] reported that some herbal medicine; *"Illicium verum*"- ethanol extract, showed antibacterial activity against 67 clinical drug-resistant isolates including *Acinetobacter baumannii, Pseudomonas aeruginosa* and Methicillin–Resistant *Staphylococcus aureus* (MRSA). Furthermore, he reported that the diethyl ether extract revealed an antibacterial activity with a minimum inhibitory concentration value of 0.15 - 0.70 $mgml^{-1}$ and 0.11 $mgml^{-1}$ respectively, and the diethyl ether extract show synergistic effect with some commercial antibiotics.

The rediscovery of the connection between plants and health is responsible for launching new generation of botanical therapeutic, multicomponent botanical drugs, dietary supplements, functional foods and plant produced recombinant proteins. The World Health Organization [5] is encouraging, promoting and

facilitating the effective use of herbal medicine in developing countries for health programs, where the potential of higher plants as a source of new drugs is still largely unexplored and hence, last decade witnessed an increase in the investigations on plants as a source of new biomolecules for human disease management [6]. Furthermore, the microbial mutations and appearances of new recombinant microorganisms necessitate the continuous assessment of the antimicrobial activities of different medicinal plants. The aim of this investigation is to asses and detects the antimicrobial agents against nosocomial infecting GIT pathogenic bacteria.

2. Materials and Methods

2.1 Screening experiment

2.1.1 Medicinal plants and extraction solvents

The active ingredients of different medicinal plants as aerial parts of *Artimesia monosperma* (Am), *Ocimum basiticum* (Ob), *Origanum majorana* L (Om), *Salvia officinalis* (So), *Pelargonium graveolens* (Pg); seeds of *Cannabis sativa* (Cs) *Foeniculum vulgare* (Fv) were extracted by different solvents viz: petroleum ether, hot water cholorform, ethyl acetate, ethanol and methanol. The ethanol gave the best result.

2.1.2 Sampling of GIT pathogenic bacteria

Culturing and identification techniques of the GIT -Samples; *Escherichia coli* (Ec), *Proteus* sp. (P), *Citrobacter* sp (c) *Shigella* sp (Sh), *Salmonella* sp (Sl) *Enterobacter* (En), *Klebsiella* sp (Kl) and *Serratia* sp (Sr) were isolated from the stool of adult patient at New (Frensh) Kasr Alainy hospital, Cairo University. Each isolate was used to inoculate on Mac Conkey and on blood agar (oxoid Corp., England).

Identification of different isolates was followed on the basis of: Gram stain, Triple sugar Iron Agar (TSI), Lysine Iron Agar Medium (LIA), Motility Indole ornithine Medium (MIO), Citrate and urease test, further morphological and biochemical schemes derived from Bergey's manual of Systematic Bacteriology [7]. Agarose gel electrophoresis of intact and treated bacterial isolates.

2.2 Sensitivity tests

2.2.1 Effect of different types of the selected antibiotics on the activities of bacterial isolates (8)

The single and combined action of some MRSA-growth non-inhibiting antibiotics (MGNIA) viz: Gentamicin (G), Cotrimoxazole (Cot), Ceftazidine (C), Spictinomycin Hcl (Spic), Ceftriaxone (Ce), Spictinomycin GD (Sp), Tobramycin (T), Cefepime (Cefe), Cefotaxime (Cef), Ceforperazone (Cefo), Amoxicillin trihydrate GE (Am) and Cefoperazone (Cefo). The single and combined action of some MRSA –growth non-inhibiting antibiotics (MGIA) Viz: Ciprofloxacin (C), Vancomycin (V), Amikacin (Ak), Ampicillin sulbactam (Amp), Kanamycin acid sulphate (K), Oxytetracyclin (Oxy), Impenem (I) and colistin sulphate (Co) and finally potentiating some MGIA and MGNIA on the growth of the pathogenic bacterial isolates of *Enterobacteriaceae* represented as mm IZD.

2.2.2 Effect of medicinal plant extracts on the activities of bacterial isolates (8).

The effect of the above mentioned plant extracts were detected on the pathogenic GIT-*Enterobacteriacae* (represented as mm IZD).

2.2.3 Medicinal plant extract-antibiotic impact experiment:

Synergistic effect of medicinal plant extract and some selected antibiotics upon different GIT-pathogenic bacterial isolates were detected [8].

1- **Investigation of volatile constituents:** Using GC/MS [9; 10 and 11].

2- **Amino acid analysis:** Separation and estimation of free amino acid pool was determined, using LC 3000 amino acid analyzer [12].

3- **Peptide content:** the procedures of [13] were followed. A sample of the borate extract was mixed with 1 ml freshly mixed in (1:l ratio) solution of 2% sodium carbonate in 4% sodium hydroxide and 0.5% copper sulphate in l% sodium tartrate. The mixture stood 10 minutes before addition of 0.1 ml folin phenol and made up to volume. The optical density of the mixture was measured, after 30 minutes, at 700 nm.

4- **Estimation of keto acids:** Keto acids were determined according to the modified method of Friedman and Haugen [14].

5- **Estimation of carbohydrate content** [15].

2.3 Statistical analysis

The experiment followed complete randomized design and the obtained data were subjected to analysis variance (ANOVA). Using Mastate programme. The least significant differences were used to compare means of treatments or probability5% [16].

3. Experimental Results

Screening experiment:

A- Impact of single and combined antibiotics upon GIT-pathogenic bacteria:

In the experiment, the following antibiotics: Gentamicin (G), Ceftazidine (C), Ceftriaxone (Ce), Cefotaxime (Cef),

Tobromycin (T), Cefoperazone (Cefo), Impenem (I), Cortimoxazole (cot), Vancomycin (V), Amikacin (Ak), Ampicillin (Amp), Cefepime (Cefe), Ciprofloxacin (C), Spictinomycin (Sp), Azithromycin (Az), Kanamycin (K), Oxytertacycline (Oxy), Colistin (Co), Spictinomycin (Spic), and Amoxycillin (Am) were examined singly or potentiated (with other one from a different group, viz MRSA-growth inhibiting antibiotics MGIA; MRSA-growth non-inhibitory antibiotics MGNIA) (Dawoud et al. 2012). The data (Table 1) have shown that applying most single/ or potentiated (MGNIA) suppressed the growth of the tested organisms viz: *E. coli* (Ec), *Proteus* sp. (P), *Citrobacter* sp. (C), *Shiegella* sp. (Sh.), *Salmonella* sp. (Sl), *Enlerobacter* sp. (En) *Klebsiella* sp (K) and *Serrati* sp.(Sr). Still some others were non-suppresser viz Amox., Cefo and their combination. Further examination of the results indicated that all the mixed MGIA suppressed the growth of the tested organisms except for Am/or Cefo. Thoroughly examination of the main antibiotic effect has shown that, mixed G+Cot gave the maximum bacterial growth inhibition (16.48 mm IZD).

Conclusion: Mixing G+Cot MGNIA antibiotics is the drug of choice for GIT-pathogen control. The main bacterial effect of resistance was insignificant except for *Proteus* was less resistant.

The data (Table 2) have shown that with a few exceptions single/or combined MGIA inhibited the growth of GIT-pathogenic bacteria, the maximum significant lethal effect was imposed by V+C (main antibiotic inhibitory effect 16.4mm IZD) followed by Ak + Amp. The least significant lethal effect was achieved by single Oxy (7.8mm IZD). The main effect of bacterial resistance was insignificant.

Conclusion: We can deduce that the lethal effect of V+C resulted only from their synergistic action. Accordingly V & C are promising in treating GIT-pathogenic bacteria. So MGIA synergize each other.

Concerning the potentiation of MGIA & MGNIA, the data (Table 3) have shown that there is a significant synergistic action between G, I resulting in the maximum main antibiotic effect (19.4mm IZD). So synergism between G, I is promising in treating GIT-pathogenic bacteria. The main effect of bacterial resistance to MGIA, MGNIA was insignificant except for *Enterobacter* and *Klebsiella* were more susceptible.

Conclusion: The data further show that mixing G & I antibiotics was the drug of choice for treating GIT-pathogenic bacteria.

So further experiment (Antibiotic & Medicinal plant impact experiments) are to be designed to differentiate the lethal effect of mixing medicinal plant extract with either of V+Cip, C+Cot or G & I antibiotics.

B- Screening experiment: Impact of antibiotics and plant extract upon GIT-pathogenic bacteria.

In the experiment the antagonistic action of the aerial part extracts (Ar E) of the following plant viz *Artimisia monosperma*(Am), *Ocimum basilicum* (Ob), *Origanum majoranum*(Or), *Salvia officinalis* (So) and *Pelargonium grandiflorum* (Pg) together with the seeds of *Foeniculum vulgare* (Fv) and *Cannabis sativa* (Cs) singly or mixed were examined upon the GIT-pathogenic bacteria.

The data of the main effect of single plant extract (Table 4) have shown the response of bacterial growth inhibition to those plant extract varied greatly where the highest significant growth inhibition was represented in (Ec), Sh&K (9.8, 10.2, 10.25mm IZD respectively) while the highest resistance to these plant extract was recorded by Sl, S and PGIT-pathogenic bacteria (5.2, 7.6, 4.2, mm IZD respectively.

Further examination of the data showed that the maximum significant antagonistic main effect was performed by Pl, and Ob(19.4 and 10.3 respectively) and the least significant one by So plants (3.5mm IZD respectively). On the other hand, the application of plant extract mixture (PEM) exhibited the highest significant antagonistic main effect (main effect 14.7mm IZD) upon different bacterial isolates except for C bacteria resistant isolate).

Conclusion

The antagonistic action of plant extract mixture (PEM) was significantly the highest (13.2mm IZD) compared with that of individual plant extracts.

Concerning the antagonistic effect of seed extract (Sd E) , the data (Table 4) further reveals that the growth of both Ec and K bacteria were adversely affected recording the maximum inhibition zone diameter (9.8 and 10.2mm IZD respectively)other bacteria were relatively resistant.

On the other hand, the main inhibitory effect of both Ca & Fv plants upon different GIT-pathogenic bacteria was mostly insignificant (6.8 and 7.8mm IZD respectively) but the main antagonistic effect of Sd Em, it was significantly higher than that of individual plant needs. A glance upon the results reveals that the total extract mixture (T E M) gave the highest significant inhibitory effect upon all GIT-pathogenic bacteria.

General conclusion: Potentiation of PEM with Sd EM (i.e. TM) reported the maximum significant main inhibitory effect on GIT-pathogenic bacteria 17.3 mm IZD).

Table (1): Single and combined action of some MRSA-growth non-inhibiting antibiotics (MGNIA) on different GIT-Pathogenic bacterial isolates of *Enterobacteriaceae* represented as (mmIZD).

Tested pathogenic Bacterial isolates / Single and combined antibiotics	*Escherichia* (Ec) *coli*	*Proteus* (P)	*Citrobacter* (C)	*Shigella* (Sh)	*Salmonella* (Sl)	*Enterobacter* (En)	*Klebsiella* (K)	*Serratia* (Sr)	Main antibiotic Effect
Gentamycin,G(10 µg)	14^{B}±0.3	12^{C}±0.2	14^{B}±0.3	13^{C}±0.3	12^{C}±0.3	12^{c}±0.3	11^{C}±0.2	10^{C}±0.2	12.3^{C}±0.3
Cotrimoxazole, Cot (25 µg)	13^{B}±0.3	14^{B} ±0.4	16^{B}±0.4	15^{B}±0.4	11^{C}±0.3	11^{C}±0.3	14^{B}±0.3	13^{C}±0.3	13.4^{C}±0.3
Gentamycin (10 µg)× Cotrimoxazole, Cot (25 µg)	16^{B}±0.4	16^{B}±0.4	18^{A}±0.4	17^{A}±0.5	15^{B}±0.5	15^{B}±0.4	17^{A}±0.4	17^{A}±0.5	16.4^{B}±0.4
Ceftazidine, C (30 µg)	-	-	-	-	-	-	-	-	-
SpictinomycinHCl, Spic (25 µg)	-	-	-	-	-	-	-	-	-
Ceftazidine (30 µg)× SpictinomycinHCl (25 µg)	14^{B}±0.3	-	14^{B}±0.5	-	16^{B}±0.5	16^{B}±0.4	-	-	7.5^{D}±0.02
Ceftriaxone, Ce (30 µg)	-	-	-	-	-	-	-	-	-
Spictinomycin GD, Sp (30 µg)	14^{B}±0.3	-	-	14^{B}±0.4	16^{B}±0.4	16^{B}±0.4	-	-	7.5^{D}±0.2
Ceftriaxone (30 µg)×Spictinomycin GD (30 µg)	18^{A}±0.4	-	-	18^{A}±0.6	18^{A}±0.6	18^{A}±0.4	14^{B}±0.4	16^{B}±0.4	12.8^{C}±0.3
Tobramycin, T (10 µg)	-	-	-	-	-	-	-	-	-
Cefepime, Cefe(30 µg)	11^{C}±0.3	10^{C}±0.2	11^{C}±0.2	11^{C}±0.2	-	-	10^{C}±0.2	10^{C}±0.2	7.9^{D}±0.2
Tobramycin (10 µg)× Cefepime(30 µg)	16^{B}±0.3	15^{B}±0.5	16±0.3	16^{B}±0.3	11^{C}±0.3	-	12^{C}±0.3	14^{B}±0.3	12.5^{C}±0.3
Cefotaxime, Cef(30 µg)	-	-	-	-	-	-	12^{C}±0.3	12^{C}±0.3	3.0^{E}±0.08
Cefoperazone, Cefo (75 µg)	-	-	-	-	-	-	-	-	-
Cefotaxime (30 µg) × Cefoperazone (75 µg)	18^{A}±0.4	18^{A}±0.5	18^{A}±0.4	17^{A}±0.4	14^{B}±0.3	14^{B}±0.3	14^{B}±0.3	14^{B}±0.3	14.6^{B}±0.3
Amoxycillintrihydrate GE, Am (25 µg)	-	-	-	-	-	-	-	-	-
Cefoperazone (75 µg), Cefo	-	-	-	-	-	-	-	-	-
Amoxycillintrihydrate GE (25 µg)× Cefoperazone (75 µg)	-	-	-	-	-	-	-	-	-
Main Effect	7.4^{D}±0.2	4.7^{E}±0.02	5.9^{D}±0.1	6.7^{D}±0.2	6.3^{D}±0.2	5.6^{D}0.2	5.8^{D}±0.3	5.9^{D}±0.3	6.0^{D}±0.3

Data with the same letters at the same column are insignificant.

Table (2): Single and combined action of some MRSA-growth inhibiting antibiotics (MGIA) on different GIT-Pathogenic bacterial isolates of *Enterobacteriaciae* represented as mmIZD.

Tested pathogenic Bacterial isolates / Single and combined antibiotics	*Escherichia (Ec) coli*	*Proteus (P)*	*Citrobacter (C)*	*Shigella (Sh)*	*Salmonella (Sl)*	*Enterobacter En*	*Klebsiella (K)*	*Serratia (Sr)*	Main antibiotic Effect
Ciprofloxacin, C(5 µg)	12^{C}±0.4	11^{C}±0.3	13^{C}±0.4	15^{B}±0.4	11^{C}±0.3	12^{C}±0.4	12^{C}±0.3	11^{C}±0.4	12.1^{C}±0.3
Vancomycin, V(30 µg)	15^{B}±0.4	15^{B}±0.4	16^{B}±0.4	17^{A}±0.4	15^{B}±0.3	15^{B}±0.3	15^{B}±0.4	14^{B}±0.4	15.3^{B}±0.4
Ciprofloxacin(5 µg) × Vancomycin(30 µg)	19^{A}±0.6	18^{A}±0.6	17^{A}±0.6	19^{A}±0.5	17^{A}±0.5	18^{A}±0.5	11^{C}±0.3	12^{C}±0.3	16.4^{B}±0.4
Amikacin, Ak (30 µg)	13^{C}±0.3	11^{C}±0.3	12^{C}±0.2	11^{C}±0.2	13^{C}±0.3	12^{C}±0.3	11^{C}±0.3	10^{C}±0.3	11.6^{C}±0.3
Ampicillin sulbactam, Amp (20 µg)	14^{B}±04	13^{C}±0.4	14^{B}±0.3	14^{B}±0.4	13^{C}±0.3	-	-	12^{C}±0.3	10^{C}±0.2
Amikacin (30 µg)× Ampicillin sulbactam(20 µg)	16^{B}±0.5	15^{B}±0.5	15^{B}±0.5	14^{B}±0.3	13^{C}±0.3	16^{B}±0.5	17^{A}±0.6	16^{B}±0.6	15.3^{B}±0.6
Kanamycin acid sulphate, K (25 µg)	11^{C}±0.2	12^{C}±0.3	11^{C}±0.2	13^{C}±0.3	-	11^{C}±0.2	12^{C}±0.2	11^{C}±0.3	10.0^{C}±0.2
Oxytetracycline, Oxy(30 µg)	11^{C}±0.2	14^{B}±0.2	-	-	14^{B}±0.3	11^{C}±0.3	-	12^{C}±0.3	7.8^{D}±0.1
Kanamycin acid sulphate (25 µg)× Oxytetracycline (30 µg)	14^{B}±0.2	16^{B}±0.4	15^{B}±0.5	13^{C}±0.5	14^{B}±0.4	14^{B}±0.6	12^{C}±0.6	14^{B}±0.4	14.0^{B}±0.4
Imipenem, I (10 µg)	10^{C}±0.2	-	10^{C}±0.2	10^{C}±0.2	11^{C}±0.2	-	-	-	5.0^{E}±0.1
Colistinsulphate, Co (30 µg)	11^{C}±0.2	10^{C}±0.2	11^{C}±0.2	-	11^{C}±0.2	11^{C}±0.3	11^{C}±0.3	-	8.1^{D}±0.1
Colistinsulphate (30 µg)× Imipenem (10 µg)	18^{A}±0.6	10^{C}±0.4	14^{B}±0.4	10^{C}±0.2	15^{B}±0.2	11^{C}±0.2	11^{C}±0.2	-	11.0^{C}±0.2
Main Effect	13.7^{C}±0.3	12.0^{C}±0.3	12.3^{C}±0.3	11.3^{C}	12.3^{C}±0.3	10.9^{C}±0.2	9.3^{C}±0.2	9.3^{C}±0.2	11.33^{C}±0.2

Data with the same letters at the same column are insignificant.

Table (3): Effect of potentiating some MGIA and/MGNIA on growth of GIT-pathogenic bacterial isolates of *Enterobacteriaciae* represented as mm IZD.

Tested pathogenic Bacterial isolates / Single and combined antibiotics	*Escherichia coli (Ec)*	*Proteus (P)*	*Citrobacter (C)*	*Shigella (Sh)*	*Salmonella (Sl)*	*Enterobacter (En)*	*Klebsiella (Kl)*	*Serratia (Sr)*	Main antibiotic Effect
Gentamycin, G (10 µg)	16^{B}±0.4	16^{B}±0.4	15^{B}±0.4	15^{B}±0.4	13^{C}±0.3	12^{C}±0.3	14^{B}±0.3	12^{C}±0.3	14.1^{B}±0.3
Imipenem, I (10 µg)	12^{C}±0.3	-	12^{C}±0.3	12^{C}±0.3	13^{C}±0.3	-	-	-	6.13^{D}±0.1
Imipenem (10 µg)×Imipenem (10 µg)	21^{A}±0.5	20^{A}±0.5	19^{A}±0.5	20^{A}±0.5	21^{A}±0.5	18^{A}±0.4	18^{A}±0.4	18^{A}±0.4	19.4^{A}±0.5
Ceftazidine, C (30 µg)	-	-	-	-	-	-	-	-	-
Vancomycin, V(30 µg)	16^{B}±0.4	16^{B}±0.4	18^{A}±0.4	19^{A}±0.4	16^{B}±0.4	18^{A}±0.4	18^{A}±0.4	18^{A}±0.4	17.4^{A}±0.4
Ceftazidine (30 µg)×Vancomycin(30 µg)	17^{A}±0.4	17^{A}±0.4	18^{A}±0.4	20^{A}±0.4	17^{A}±0.3	19^{A}±0.4	19^{A}±0.4	19^{A}±0.4	18.3^{A}±0.4
Ceftriaxone, Ce (30 µg)	-	-	-	-	-	-	-	-	-
Amikacin, Ak (30 µg)	14^{B}±0.3	13^{C}±0.3	13^{C}±0.3	12^{C}±0.3	12^{C}±0.3	12^{C}±0.3	13^{C}±0.3	13^{C}±0.3	12.8^{C}±0.5
Ceftriaxone (30 µg)×Amikacin (30 µg)	16^{B}±0.3	13^{C}±0.3	13^{C}±0.3	13^{C}±0.4	15^{B}±0.2	13^{C}±0.4	14^{B}±0.4	14^{B}±0.4	13.9^{C}±0.4
Cefotaxime, Cef(30 µg)	-	-	-	-	-	-	12^{C}±0.3	12^{C}±0.3	3.0^{E}±0.1
Ampicillin sulbactam, Amp (20 µg)	16^{B}±0.4	15^{B}±0.4	16^{B}±0.4	14^{B}±0.3	13^{C}±0.3	-	-	12^{C}±0.7	10.8^{C}±0.3
Cefotaxime(30 µg)× Ampicillin sulbactam(20 µg)	17^{A}±0.4	16^{B}±0.4	17^{A}±0.4	15^{B}±0.4	13^{C}±04	-	-	14^{B}±0.4	11.5^{C}±0.4
Tobramycin, T (10 µg)	-	-	-	-	-	-	-	-	-
Ciprofloxacin, Cip(5 µg)	14^{B}±0.4	15^{B}±0.4	14^{B}±0.3	15^{B}±0.3	13^{C}±0.3	11^{C}±0.3	12^{C}±0.2	12^{C}±0.2	13.1^{C}±0.4
Tobramycin (10 µg))× Ciprofloxacin(5 µg)	15^{B}±0.4	16^{B}±0.4	15^{B}±0.3	15^{B}±0.3	14^{B}±0.3	12^{C}±0.3	12^{C}±0.2	12^{C}±0.2	13.9^{C}±0.4
Cefoperazone, Cefo (75 µg)	-	-	-	-	-	-	-	-	-
Kanamycin acidsulphate, K (25 µg)	12^{C}±0.3	13^{C}±0.3	14^{B}±0.3	14^{B}±0.3	-	11^{C}±0.3	12^{C}±0.3	11^{C}±0.3	13.9^{C}±0.4
Cefoperazone (75 µg)×Kanamycin acid	13^{C}±0.3	13^{C}±0.3	15^{B}±0.3	15^{B}±0.3	-	12^{C}±0.3	13^{C}±0.4	12^{C}±0.4	11.6^{C}±0.3
Cefepime, Cefe(30 µg)	12^{C}±0.3	12^{C}±0.3	11^{C}±0.2	11^{C}±0.2	11^{C}±0.2	12^{C}±0.2	12^{C}±0.3	12^{C}±0.3	11.6^{C}±0.3
Oxytetracycline, Oxy(30 µg)	13^{C}±0.4	14^{B}±0.4	-	-	14^{B}±0.3	11^{C}±0.3	-	12^{C}±0.3	8.0^{D}±0.1
Cefepime(30 µg)×Oxytetracycline 30 µg)	19^{A}±0.5	20^{A}±0.4	15^{B}±0.4	15^{B}±0.4	16^{B}±0.3	16^{B}±0.3	14^{B}±0.3	16^{B}±0.4	16.4^{B}±0.5
Spictinomycin GD, Spic Gd (30 µg)	-	-	-	15^{B}±0.3	15^{B}±0.3	-	-	-	3.8^{E}±0.1
Colistinsulphate, Co (30 µg)	16^{B}±0.5	16^{B}±0.3	11^{C}±0.3	-	11^{C}±0.3	14^{B}±0.4	12^{C}±0.4	-	10.0^{C}±0.2
Spictinomycin GD (30 µg)× Colistinsulphate (30 µg)	18^{A}±0.4	18^{A}±0.4	14^{B}±0.3	20^{A}±0.4	20^{A}±0.4	16^{B}±0.3	16^{B}±0.4	-	15.3^{B}±0.4
Azithromycin dehydrate , Az(25 µg)	-	-	-	-	-	-	-		-
Ampicillin sulbactam, Amp (20 µg)	16^{B}±0.3	15^{B}±0.3	16^{B}±0.3	14^{B}±0.4	13^{C}±0.3	-	-	12^{C}±0.5	10.8^{C}±0.2
Azithromycin dehydrate (25 µg)× Ampicillin sulbactam(20 µg)	20^{A}±0.5	19^{A}±0.5	20^{A}±0.5	18^{A}±0.5	19^{A}±0.3	-	18^{A}±0.4	19^{A}±0.4	16.7^{B}±0.4
Main effect	11.6^{C}±0.3	11.0^{C}±0.3	10.6^{C}0.2	10.8^{C}±0.2	10.3^{C}±0.2	7.7^{D}±0.1	8.5^{D}±0.1	9.3^{C}±0.2	10.0^{C}

Data with the same letters at the same column are insignificant

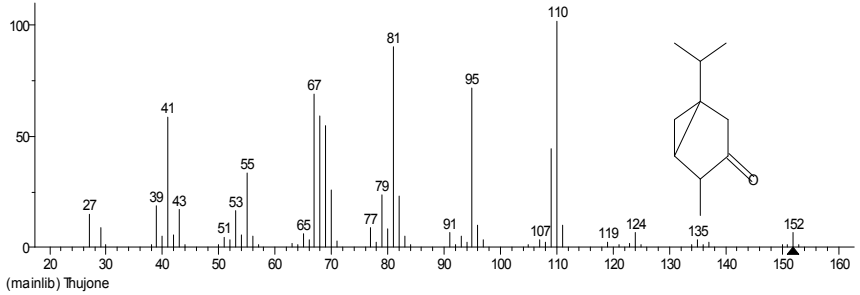
Table (4): Effect of different plant extract on growth of GIT-pathogenic bacterial isolates of *Enterobacteriaciae* represented as mm. IZD.

Tested pathogenic Bacterial isolates / Single and combined antibiotics		*Escherichia coli (Ec)*	*Proteus (P)*	*Citrobacter (C)*	*Shigella (Sh)*	*Salmonella (Sl)*	*Enterobacter (En)*	*Klebsiella (Kl)*	*Serratia (Sr)*	*Main effect of bacteria*	*Major essential oil compound /plant*
Aerial parts	*Artimesia monosperma* (Am)	$12.0^{B}\pm0.3$	-	-	$13.0^{B}\pm0.4$	-	$14.0^{A}\pm0.4$	$14.5^{A}\pm0.4$	-	$5.6^{D}\pm0.1$	α-pinene, β-pinene,Stragol
	Ocimumbasilicum (Ob)	$13.0^{B}\pm0.4$	$10.0^{B}\pm0.3$	-	$12.5^{B}\pm0.3$	$12.0^{B}\pm0.3$	$13.0^{B}\pm0.4$	$12.5^{B}\pm0.4$	$12.0^{B}\pm0.4$	$10.3^{B}\pm0.3$	α-pinene, β-pinene γ-terpinene, γ-terpinolene, Eugenol
	Origanum majorana L (Om)	$12.0^{B}\pm0.4$	-	-1	$12.0^{B}\pm0.3$	$14.0^{A}\pm0.4$	-	-	-	$5.40^{D}\pm0.2$	Caryophyllene
	Salvia officinalis L (So)	-	-	-	-	-	$13.0^{B}\pm0.3$	$12.5^{B}\pm0.3$	$12.0^{B}\pm0.3$	$3.5^{D}\pm0.1$	Thugen
	Pelrgonium graveolens (Pg)	$12.0^{B}\pm0.3$	$11.0^{B}\pm0.3$	-	$14.0^{A}\pm0.4$	-	$13.0^{B}\pm0.4$	$13.0^{B}\pm0.4$	$14.0^{A}\pm0.4$	$9.14^{C}\pm0.3$	α-pinene, β-pinene, γ-terpinene
	Main effect of plants	$9.8^{B}\pm0.3$	$4.2^{D}\pm0.1$	-	$10.3^{B}\pm0.3$	$5.2^{D}\pm0.2$	$11.0^{B}\pm0.3$	$10.2^{B}\pm0.3$	$7.6^{C}\pm0.2$	$7.00^{C}\pm0.2$	α-pinene, β-pinene, Stragol, γ-terpinene, γ-terpinolene, Caryophyllene, Thugen, Eugenol
	Mixture of plants (PM)	$16.0^{A}\pm0.4$	$14.0^{A}\pm0.4$	-	$15.0^{A}\pm0.4$	$16.0^{A}\pm0.4$	$15.0^{A}\pm0.5$	$16.0^{A}\pm0.4$	$15.0^{A}\pm0.4$	$13.2^{B}\pm0.3$	α-pinene, β-pinene, Stragol, γ-terpinene, γ-terpinolene, Caryophyllene, Thugen, Eugenol
Seeds (Sd)	*Canvabis sativa* (Cs)	$14.0^{A}\pm0.3$	-	-	$14.5^{A}\pm0.3$	-	$10.0^{B}\pm0.2$	-	-	$4.07^{D}\pm0.2$	α-pinene,β-pinene,γ-terpinene, Geranyllinolool
	Foeniculum vulgare (Fv)	$10.0^{B}\pm0.3$	$11.0^{B}\pm0.3$	$11.0^{B}\pm0.3$	-	-	$10.0^{B}\pm0.3$	$10.0^{B}\pm0.3$	$13.0^{B}\pm0.3$	$7.8^{C}\pm0.2$	α-pinene,β-pinene, Fenchon, Limonene
	Main effect of seeds	$12.0^{B}\pm0.3$	$5.5^{D}\pm0.1$	$5.5^{D}\pm0.1$	$7.5^{C}\pm0.2$	-	$12.0^{B}\pm0.3$	$11.5^{B}\pm0.3$	$6.5^{C}\pm0.2$	$6.8^{CD}\pm0.2$	α-pinene, β-pinene, Stragol, γ-terpinene, Geranyllinolool, Fenchon, Limonene
	Mixture seeds (SM)	$16.0^{A}\pm0.4$	$12.0^{B}\pm0.3$	$11.0^{B}\pm0.3$	$14.5^{A}\pm0.3$	-	$12.0^{B}\pm0.3$	$12.0^{B}\pm0.3$	$18.0^{A}\pm0.4$	$11.9^{B}\pm0.3$	α-pinene, β-pinene, Stragol, γ-terpinene, Geranyllinolool, Fenchon, Limonene

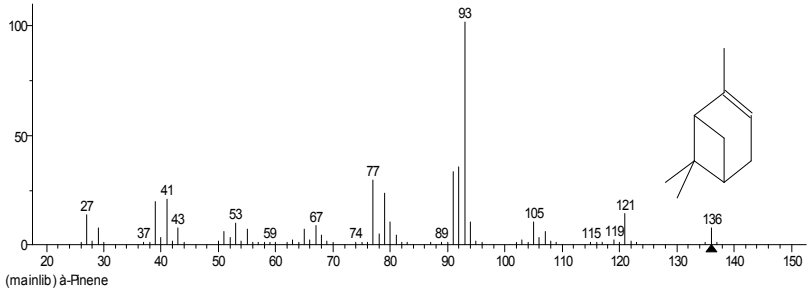
Tested pathogenic Bacterial isolates / Single and combined antibiotics	*Escherichia coli (Ec)*	*Proteus (P)*	*Citrobacter (C)*	*Shigella (Sh)*	*Salmonella (Sl)*	*Enterobacter (En)*	*Klebsiella (Kl)*	*Serratia (Sr)*	*Main effect of bacteria*	*Major essential oil compound /plant*
Mean effect	13^{B}±0.3	7. 1^{C}±0.2	6.9^{C}±0.2	9.1^{B}±0.2	-	11.0^{B}±0.3	8.4^{B}±0.2	9.4^{B}±0.3	7.6^{C}±0.2	
Mixture of plant + seeds (TM)	20.0^{A}±0.4	16.0^{A}±0.4	18.0^{A}±0.5	19^{A}±0.4	16.0^{A}±0.4	16.0^{A}±0.4	17.0^{A}±0.4	15.0^{A}±0.4	17.3^{A}±0.4	α-pinene, β-pinene, Stragol, γ-terpinene, γ-terpinolene, Caryophyllene, Thugen, Geranyl linolool, Fenchon, Limonene, Eugenol

GC/MS fragmentation and structural of essential oil of studies plants:

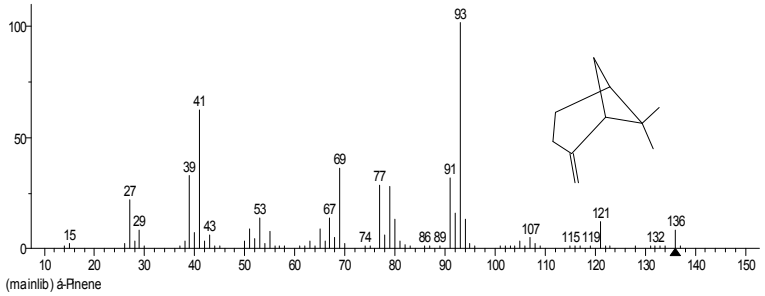
α – thugen MW 152



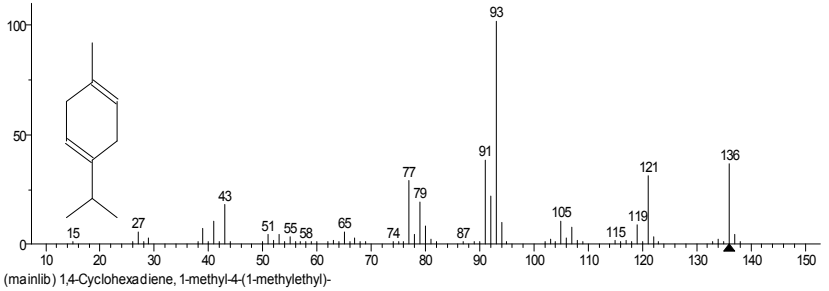
α – pinene MW



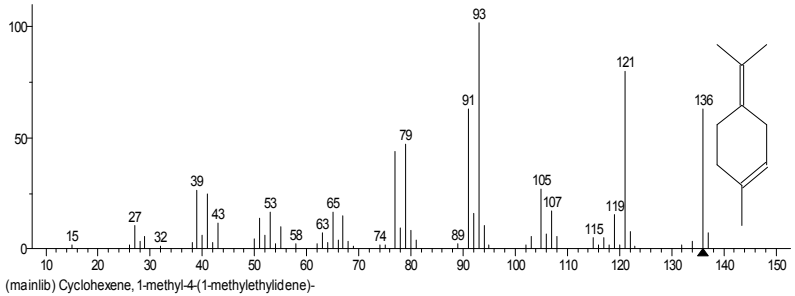
β-pinene MW 136



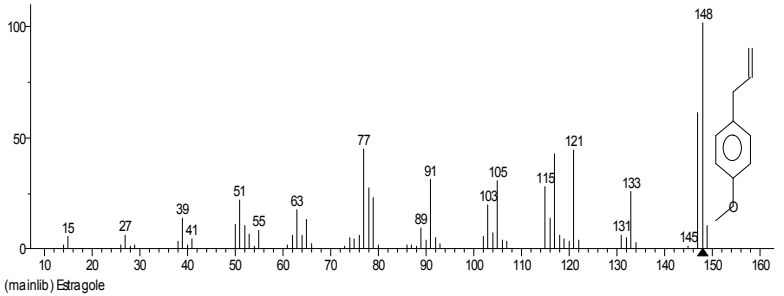
γ – terpinene MW 136



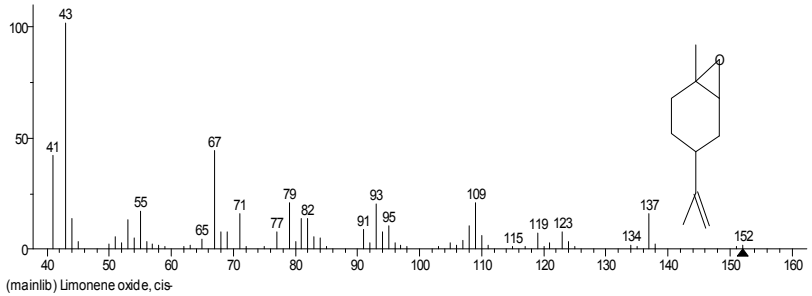
α – terpinolene MW 136



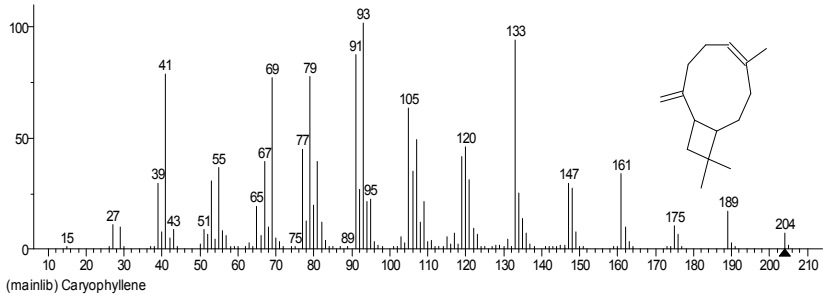
Stragole MW 148



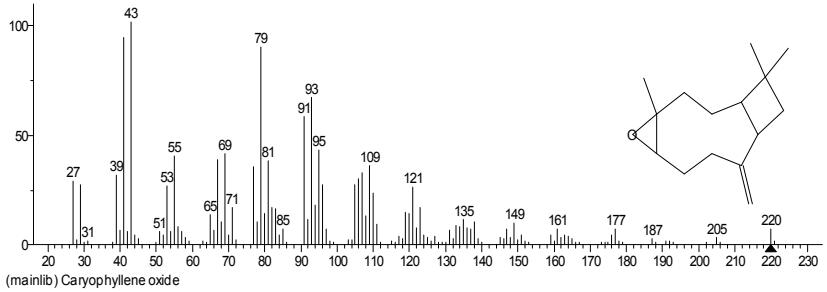
Cis-limonene oxide MW152



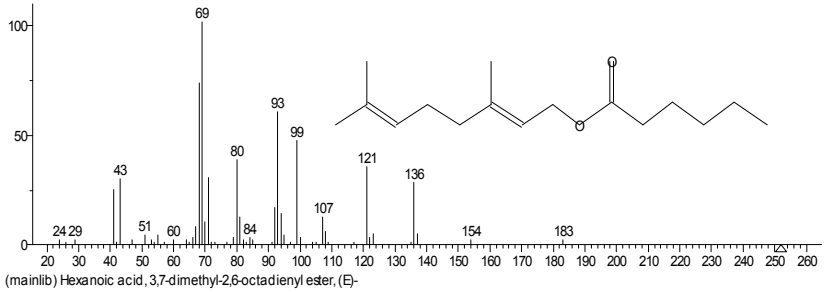
β- caryophyllene MW 204



caryophylleneoxideMW 220



GeranylhexanoateMW 252



GC/MS essential oil analysis: GC/MC analyses have shown that the most dominant major oils were (1-10) and Table 4.

II- Antibiotic-Medicinal Plant Mixture Impact Experiment

The data (Table 5) have shown that G+I antibiotics give the maximum significant inhibition zone diameter photo 1 (25mmIZD) while V+Cip mixed with plant extract gave the least significant inhibition zone photo 3(18 mm IZD) and that of C+Cot antibiotics mixed with plant extract had an intermediate inhibitory effect (21.5 mm IZD photo 3) on GIT-pathogenic bacteria on the other hand, the response of different bacteria to the same treatment was insignificantly different.

General conclusion: the most effective drugs for inhibition of pathogenic GIT bacterial growth was G+I+TM (mixture of plant extract, GITM).

On studying the metabolism of different GIT-pathogenic bacteria under the impact of (GITM) the data (Table 6) have shown that the bacterial cell permeability to the synthesized protein components (% control of extracellular amino acids and peptides increased greatly recording 12.5% control Amino acids/C, 119% control peptides/K. on the contrary , there is a great drop of the percentage control of cellular component of amino acid, peptides and proteins reflecting the inhibition of protein synthesis, deposition of cellular keto acids and leakage of the membrane to the building blocks of protein across the cellular membrane due to it adverse damage (Table 6).

Conclusion GITM inhibit protein synthesis and destroy cellular membrane of GIT-pathogenic bacteria.

Discussion

Enterobacteriaceae or intestinal bacilli grow profusely on simple media such as peptone water and plain nutrient agar and forms colonies on MacConkey's medium e.g *Escherichia coli* (Ec), *Klebsiella* (K), *Salmonella* (Sl), *Shigella* (SH), *Proteus* (P), *Citrobacter* (C), *Serratia* (S) and *Enterobacter* (E). These bacteria cause many diseases as gastroenteritis, respiratory & urinary tract infections, food poisoning, typhoid fever, septicemia, and Bacillary dysentery, some are resistant to many of the commonly used antibiotics and is liable to cause super infection during antibiotic therapy [17]. So, the objective of the study is to search for combined effective safe drugs for the control of pathogenic GIT-*Enterobacteriaceae*.

On the other hand, application of single antibiotic may be disappointed for disease control, so a combination of more than one antibiotic (or formulation of antibiotics with other drugs) may have a crucial lethal effect on the above mentioned GIT-pathogenic *Enterobacteriaceae.* In this connection, it was stated that the treatment of 17 patients with suspected postoperative endophthalmitis with 0.2 mg vancomycin and 0.05 mg Gentamicin intravitreally, there were adequate intravitreal vancomycin and gentamicin concentration for over a week, there were no adverse effect [18]. Furthermore, [4] reported that some herbal medicine *Lllocium verum* ethanol extract showed antibacterial activity against 67 climical drug-resistant isolates including *Acinetobacter baiemannii, Pseudomonas aeruginosa* and 20 methicillin-resistant *Staphylocccus aureus*.

In screening experiment, the results (Table 1, Fig 1) have shown that Gentamicin (Amino glycosides) mixed with cotrimoxazole (Trimethoprim combination) gave the highest lethal effect on bacterial growth (16.4±0.4mm IZD) these was due to the fact that cotrimoxazole (Bactrim, Eusparim, Septrin) contain methoprim and sulfamethoxazole that is bactericidal which inhibit dihydrofolate reductase causing partial sequencal blockade leading to defective protein synthesis and cytoplasmic damage that in turn results in marked increase in their uptake [19]. Concerning the Gentamicin antibiotic, it was stated that Gentamicin is well established for the treatment of several bacterial infections, especially those caused by Gram-negative bacteria, including *Pseudomonas aerignosa, Klebsiella* species and *Serratia marcescens* [20] on the other hand, Gentamicin and other aminoglycosides have increased activity when they are combined. The proposed mechanism of their synergism is damage to cell membrane followed by improved diffusion of gentamicin across the outer bacterial membrane [21].

Furthermore, the data (Table 2 and Fig. 2) have showed that mixing the growth mersa Inhibiting antibiotics (MGIA) vancomycin and ciprofloxacin gave the maximum lethal effect on bacterial grow (16.4 mm IZD). Vancomycin is a narrow spectrum glycopeptide antibiotic with potent antistaphylococcal activity [22]. Vancomycin is rarely associated with serious toxicity vancomycin inhibits bacterial cell wall synthesis and bactericidal during cell division. Vancomycin- resistant *Enterococci* have been recovered with increasing frequency in hospitalized patients. Judicious use of vancomycin and broad spectrum antibiotics is recommended and strict infection control measures must be implemented to prevent nosocomial transmission of these organisms [23]. For Ciprofloxacin (fluoroquinolone) is an antibacterial drug with a wider spectrum. Antimicrobial prophyloxis to prevent inhalational anthrax has been recommended for people potentially exposed to *Bacillus anthracis* [24] so we can conclude that, the lethal effect of vancomycin resulted from synergism with Ciprofloxacin.

Table (5): Impact of mixing dual antibiotic with medicinal extract upon the growth of GIT-pathogenic bacteria isolates of *Enterobacteriaciae* represented as mm. IZD.

GIT-pathogenic mixed bacteria antibiotics / mixed medicinal plant extract + Antibiotics	*Eschericia coli (Ec)*	*Proteus (P)*	*Citrobacter (C)*	*Shigella (Sh)*	*Salmonella (Sl)*	*Enterobacter (En)*	*Klebsiella (Kl)*	*Serratia (Sr)*	Main effect
Vancomycin, V(30 µg)+ Ciprofloxacin, Cip (5 µg)+ mixture of plant extract (TM)	18^{C} ±0.6	19^{C}±0.6	20^{B}±0.4	18^{C}±0.4	20^{B}±0.6	16^{C}±0.6	16^{C}±0.4	17^{C}±0.4	18.0^{C}±0.4
Gentamycin, G (10 µg)+Cotrimoxazole, Cot (25 µg) +mixture of plant extract (TM)	23^{AB}±0.5	23^{AB}±0.4	23^{AB}±0.6	22^{B}±0.5	20^{B}±0.4	20^{B}±0.6	20^{B}±0.6	21^{B}±0.4	21.5^{B}±0.4
Gentamycin, G (10 µg)+Imipenem, I (10 µg)+ mixture of plant extract (TM)	26^{A}±0.6	26^{A}±0.9	25^{A}±0.6	25^{A}±0.5	26^{A}±0.7	24^{A}±0.6	24^{A}±0.8	24^{A}±0.6	25.0^{A}±0.5
Main effect	22.3^{B}±0.1	22.6^{B}±0.5	22.6^{B}±0.5	21.6^{B}±0.5	22.0^{B}±0.5	20.0^{B}±0.5	20.0^{B}±0.4	20.6^{B}±0.4	21.3^{B}±0.5

Data with the same litter at the same column are insignificant

Table (6): GIT-pathogenic bacterial metabolism in response to (GITM) mixture (represented as % control)

Gentamycin (10 µg)+Imipenem (10 µg)+TM+Pathogenic bacteria	% Control protein compound					% control carbon compounds		
	Extracellular		Cellular			Extracellular		
	Amino acids %	Peptides	Amino acids	Peptide	Protein	Residual carbohydrate	Keto Acids	Cellular Keto Acids
Eschericia coli(Ec)	120^{A}±6.0	110^{A}±5.0	10^{C}±0.1	41^{B}±0.8	37^{A}±30	200^{A}±8.0	96^{A}±4	140^{B}±7.0
Proteus (P)	118^{A}±7.0	108^{A}±6.0	13^{C}±0.1	39^{AB}±0.8	41^{A}±3.0	198^{A}±7	99^{A}±5	122^{Cd}±8
Citrobacter (C)	125^{A}±6.0	107^{A}±7.0	15^{C}±0.2	42^{B}±0.9	40^{A}±4.0	187^{B}±8	92^{A}+6	117^{D}±8
Shigella (Sh)	124^{A}±6.0	114^{A}±5.0	20^{B}±0.2	50^{A}±0.9	39^{A}±3.0	191^{A}±9	91^{A}±4	129^{C}±7
Salmonella (Sl)	108^{A}±8.0	111^{A}±4.0	10^{C}±0.2	51^{A}±0.9	38^{A}±4.0	188^{B}±7	89^{B}±5	133^{C}±9
Enterobacter(En)	121^{A}±8.0	113^{A}±4.0	16^{C}±0.2	38^{AB}±0.8	38^{A}±4.0	178^{B}±8	88^{B}±6	135^{B}±9
Klebsiella (Kl)	123^{A}±7.0	119^{A}±4.0	19^{BC}±0.2	33^{C}±0.8	41^{A}±4.0	187^{B}±9	92^{A}±6	141^{B}±11
Serratia (Sr)	119^{A}±9.0	109^{A}±4.0	30^{A}±0.3	37^{C}±0.8	42^{A}±4.0	189^{B}±7	92^{A}±7	163^{A}±8.0
Main effect	119.8^{A}±9	111.4^{A}±6	16.6^{C}±30.4	41.4^{B}±2.0	39.5^{A}±3.9	189.0^{B}±8.0	92.4^{A}±9	136^{B}±9

Data with the same letter at the same column are insignificant

Moreover, the data (Table 3, Fig. 3) further show that, also gentamicin (MGNIA) combined with Imipenem (MGIA) has the most lethal effect on GIT-pathogenic *Enterobacteriaceae* due to its functions on bacterial cells. They cause misreading of the RNA code/or inhibition of the polymerization of the amino acids [25]. Concerning the response of the different bacterial isolates, the main susceptibility effect was mostly insignificant this may be due to the similar gram negative cell wall structure and the antigenic characteristics of the *Enterobacteriaceae* members.

In the second screening experiment, the selected combined antibiotics vancomycin & ciprofloxacin; Gentamycin & cotrimoxazole and Gentamycin & Impenem were further mixed with the selected plant to detect the favourable drug for controlling pathogenic GIT-bacteria in screening experiment B, the susceptibility of Medicinal plants (*Artmesia monosperma* (Am), *Ocimum basilicum* (Ob), *Origanum majorana* (Om), *Savia officinalis* (So), *Pelargonium graveolens* (Pg) and their mixture (PM). Also the seed extract of *Cannabis sativa* (Cs), *Foeniculum vulgare* (Fv), and their mixture (SM), furthermore, the plant (PM) mixture and seed mixture™. The maximum main effect (17.3 mm IZD) was recorded under the effect of PM+SM i.e (TM), these mixtures contains the most dominant essential oil ingradients (α-pinene, Stragol, γ terpinene, Geranyl limolool, Fenchon and Limonene. In this connection [26] stated that, the essential oil of ripe fruit of *S. terebinthifolius* Raddi (Limonene, α pinene, transcaryophyllene , α-phellandrene) was active against wild strain of hospital origin (*E. coli, pseudomonas* sp., *Klebsiella oxytoca Corynebacterium* sp. *Staphylococcus aureus, Enterobacter, Nocardias* and *Sterptococcus* group D. Concerning the seeds and aerial plant parts which may be effective more than antibiotic, [27] recorded that the essential oil composition of leaves and fatty acids from the seeds (of *sesbania punicea, Rottlebox*) as cineole, α-pinene, linoleic acid and oleic acid. Antibacterial activity of ethanol extract was evaluated against 8 gr (+/-) bacteria from which *Salmonella partyphi* B was the most sensitive one, even more than chloramphenicol as a standard antibiotic.

Diao *et al.,* [28] stated that *Amomum kravanh* (culinary spice) contains cincoli, α-pinene α-terpinene and α-terpinene, and β-pinene essentials oils that was inhibitory to *Bacillus,* a gram positive bacterium; *E coli* gram negative bacterium and observation of cell membrane permeability disruption, cell constituent release assay, and transmission electron microscopy indicated that this essential oil may disrupt the cell wall and cell membrane permeability, leading to leakage of intracellular constituent in both *B. subtilis* and *E.coli* and was most active against six bacterial strains including *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, E. coli* and *Klebsiella peneumoniae, O. Syriacum* was also the most active plant against all *Candida* strains with inhibition zone ranged from 22-5-29.5mm.

In our experiment the maximum inhibition zone (25.0, table 5)under the antagonistic effect of Antibiotics Gentamycin combined with Impenenm potentiated with total plant mixture TM is due to the cumulative and synergistic effect of these ingredient i.e damage of cell wall by Gentamicin [29]; Impenenm causes misreading of RNA code /or inhibition of the polymerization of amino acids [25]. Also essential oils may disrupt the cell wall and cell membranes permeability leading to leakage of intracellular constituents [28]. These effects are confirmed by the data (Table 6) which indicate the hydrolysis of protein and its outward diffusion and blocking of amination of keto acids.

General conclusion: combination of Gentamicin, Impenem and TM are promising in crucial treatment of GIT-pathogenic bacteria.

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