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The Combination Between Some Medical Oils Antibiotics and Its Effect on Some Pathogenic Microorganisms

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Abstract

For many years, the battle between humans continues and the multiplicity of infections and disease-causing diseases. Emerging on the battlefield as one of the most important challenges facing human health is bacterial resistance and its rapid rise. These have become a major global public health concern revitalizing the need for new antimicrobial compounds. A rational approach to dealing with problems of antibiotic resistance requires detailed knowledge of the various biological and non-biological factors that influence the rate and extent of resistance development. A combination therapy combining traditional antibiotics and essential oils is currently blooming and represents a potential area for future investigations. This new generation of phytopharmaceutical may shed light on the development of novel drug regimens in the fight against antibiotic resistance. This review reinforced and described the results of observed synergies between essential oils and antibiotics, and highlighted the potential of essential oils as a potential resistance modifying agent. The aim of this thesis is to verify whether the bio-reactivity of medicinal oils can be assessed using microorganisms in standard and clinical testing and their sensitivity to specific antibiotics. The bacteria which produce antibiotics must themselves be resistant to those antibiotics or they would kill themselves. Other bacteria can also become resistant to the antibiotics if they are exposed to the antibiotics for long periods or at concentrations that are not adequate to kill the microorganisms immediately [1]. Disease producing bacteria which infect humans and are resistant to one, or multiple, antibiotics are a serious problem for health care in many parts of the world. For this reason, new antibiotics must be discovered, approved, and made available for medical use continuously. The study was performed on 5 isolated bacterial samples from different medical laboratories and one samble from yeast. These samples were tested bacteriologically and the samples were grown under ideal conditions under anaerobic and anaerobic conditions. The ability of eight natural lemon oils with different antibiotics to kill antibiotic-resistant bacteria, which are onion, garlic, watercress, rosemary, thyme, parsley, mint and cinnamon, were tested with the following antibiotics, vancomycin, chloramphenicol, ofloxacin, ampicillin, amoxicillin, moxifloxacin, ampicillin, amoxicillin, and trafloxacin. The results showed that E. coli bacteria are very sensitive to all oils in the case of all antibiotics, especially parsley oil, which is the most efficient oil in affecting the isolated bacteria. and Identification was confirmed for isolates Candida albicans strain, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes. The most resistant to antibiotics after making molecular studies by studying its genetic sequence and it was registered in the International Genebank.

1. Introduction

Microbiology is the study of microorganisms, which are unicellular and are seen only under a microscope. This includes prokaryotes like viruses and eukaryotes like certain algae, fungi, and protozoans. Microbiology is a branch of biology and deals with much sub-branches-like Virology, Bacteriology, Mycology, and Parasitology

More than half of our antibiotics are produced by species of Streptomyces. They are filamentous bacteria that commonly inhabit the soil. Few antibiotics are produced by molds, mostly of the genera Penicillium and cephalosporins. With the advent of organic chemistry, many antibiotics are now obtained by chemical synthesis. Sulfa drugs are synthetic.

Mechanisms of action and resistance of antibiotics

The mechanism of action of antimicrobial agents can be categorized based on the function that is affected by the agents, these generally included the following: inhibition of the cell wall synthesis, or nucleic acid synthesis, inhibition of ribosome function, or cell membrane function and inhibition of folate metabolism.

Antibiotic Resistance

Antibiotic resistance is the ability of bacteria or other microbes to resist the effects of an antibiotic Fig (1). Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs [4].

The bacteria survive and continue to multiply causing more harm. It is a specific type of drug resistance. The emergence of bacterial strains that are resistant to current antibiotics is an important public health concern. Antibiotic resistance evolves naturally via natural selection through random mutation. Once such a gene is generated, bacteria can then horizontally transfer the genetic information (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multi-resistant.

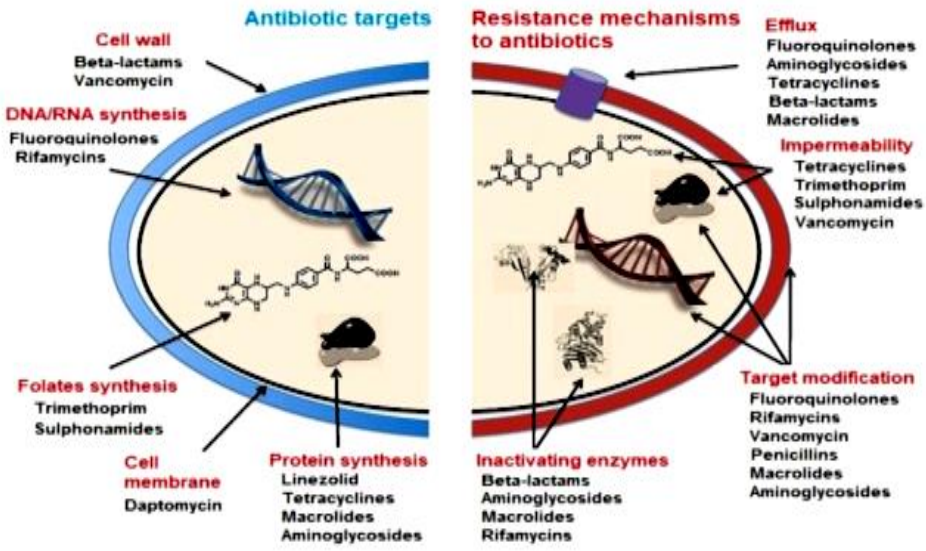

Fig (1) Antibiotic resistance.

2. Materials and methods

This study was conducted on different bacterial samples resistant to different types of antibiotics. The samples were collected from different clinical labs. Study was done from November, 2019 till July, 2020.

2.1 Sample collection and processing

The specimens were collected and transported according to (Murray et al., 2007) under aseptic conditions directly to the

Microbiology Laboratory at Clinical department Genetic Engineering Research Department (VACSERA) (Cairo, Egypt) where the study was carried out.

2.2 Isolation and cultivation of bacteria

A 1 cm2 area of clinical samples was collected with a sterile loop of and then inserted into broth tube as transport media. The contents were collected under complete aseptic condition. Samples were cultured:

Bacterial isolates: samples were inoculated on Trypticase soya agar plate for 24 hrs at 37˚ C.

Yeast isolates: samples were inoculated on Sabroud dextrose agar plate for 48 hrs at 28˚ C. 3.3. Media used for Isolation and cultivation of bacteria:

Trypticase soya agar [5].

Tryptic Soy Agar is used as a general growth medium for the isolation and cultivation of microorganisms. They are general-purpose, non-selective media providing enough nutrients to allow for a wide variety of microorganisms to grow. This medium is also recommended for use in the cultivation, storage, maintenance and transportation of pure cultures of microorganisms. 17 gm pancreatic digest of casin , 3 gm papaic digest of soyabean meal, sodium chloride 5gm, dextrose 2.5 gm, 2.5 dipotasium hydrogen phosphate and 15gm agar were suspended in one liter of distilled water then dissolved and boiled. Autoclave at 121 ˚C for 15 min, cool to 40-50 ˚C then added 5% blood were added under sterile condition. PH was adjusted to 7.3 ± 0.2 at 25oC by using 0.1N HCl or 0.1 N NaOH before sterilization.

Sabroud dextrose agar

Sabouraud Dextrose Agar (SDA) is used for the isolation, cultivation, and maintenance of non-pathogenic and pathogenic species of fungi and yeasts. SDA was formulated by Sabouraud in 1892 for culturing dermatophytes. The pH is adjusted to approximately 5.6 in order to enhance the growth of fungi, especially dermatophytes, and to slightly inhibit bacterial growth in clinical specimens.

40 gm dextrose, 10 gm peptone and 15gm agar were suspended in one liter of distilled water then dissolved and boiled. Autoclave at 121 ˚C for 15 min, cool to 40-50 ˚C then added 5% blood were added under sterile condition. PH was adjusted to 5.6 ± 0.2 at 25^{o}C by using 0.1N HCl or 0.1 N NaOH before sterilization.

2.3 Identification of bacterial isolates

Bacterial isolates were identified according to Bergey's Manual of determinative bacteriology.

The colonies morphology

Colony size, shape, whether it is opaque or translucent, mucoid or dry production of characteristic colony color or any change in the color of the agar.

Microscopic examination

To differentiate between Gram-positive (purple or violet color) and Gram-negative (red or pink color). A heat fixed smear from 18-24 hrs bacterial culture was prepared on a clean slide. Smears were stained with crystal violet solution for 30 sec, and then

washed with distilled water. The prepared smear was covered with Gram's iodine solution for 10 sec.

The iodine was poured off and slide was washed with a decolorized (mixture of ethanol and acetone 95 %) until no more purple stain runs from the slide (10-20 sec). The slide was gently washed with distilled water, then stained with counter stain (safranin) for 30 sec, then washed with distilled water and blot dry. The dried slides were microscopically examined under the oil immersion lens.

2.5 16S rRNA/ 18S rRNA genessequencing

Partial 16S rRNA gene sequencing was used to confirm the biochemical identification.

Universal 16s rRNA primers 27 f (5'-AGAGTTTGATCMTGGCTCAG-3').

Molecular identification of the isolate strains

The identification of the most resistant and frequent strain, Staphylococcus epidermidis and staphylococcus aureus, …….. were confirmed by investigation of 16S r RNA gene sequences using a 310 genetic analyzer sequencer. Selected Bacterial DNA was extracted using GenEluteTM Bacterial Genomic DNA Kit (Sigma Aldrich) as per manufacturer protocol. Amplicons of 16S rRNA were sequenced in (VACSERA) Egypt, using a 310 genetic DNA Sequencer (Genetic Analyzer, Applied Biosystems, USA).

Chemicals and reagents

All reagents used were of analytical grade. PCR and sequencing chemicals, molecular biology kits used were purchased from (Local supplier).

Extraction and purification of bacterial genomic DNA

The DNA of the selected bacteria were extracted using GenEluteTM Bacterial Genomic DNA Kit, Sigma Aldrich as per manufacturer protocol and can be summarized as following: After 24 h growth at 37 ˚ C on LB media, bacterial cells were centrifuged for 10 min at 12000 rpm at 4˚C. The supernatant (growth medium) was discarded and the bacterial pellet was resuspended in 400 µL of lysis buffer containing 50 µL of lysozyme and 25 µL of RNase then, incubation at 37˚C for 15 min. With careful shaking. 50µL of 20% lauroylsarcosine was added to the lysates, kept on ice for 5min to obtain a translucent material.

3. Results

Screening of antibacterial activities of different essential oils

The antimicrobial activity of a selection of eleven Egyptian essential oils against the isolated bacteria was investigated and the results are summarized in Tables (1-6). Over and done with this experiment eight essential oils were nominated for carrying out the antimicrobial susceptibility test by Cinnamon Oil, Rosemary Oil, Thyme Oil, Parsley Oil, Onion Oil, Garlic Oil, Arugula oil, and mint oil along with different antibiotic against all tested bacterial species Fig (1) and Sch (1).

Table (1) Antimicrobial activity of different essential oils on the isolated bacteria vs. Chloramphenicol antibiotic.

Bacteria OSE	E. coli	Staph. aureus	Pseudomonas sp.	Streptococcus sp.	Klebsiella sp.	Candida albicans
Control	21	25	20	22	30	24
Onion	24	26	25	23	31	27
Garlic	24	27	24	22	25	25
Arugala	23	27	26	24	32	26
Rosemary	24	25	25	20	28	25
Thyme	22	26	25	21	31	27
Parsley	22	28	25	20	32	26
Mint	23	25	20	18	29	24
Cinnamon	25	26	24	22	28	25

Table (2) Antimicrobial activity of different essential oils on the isolated bacteria vs. OX antibiotic.

Bacteria OSE	E. coli	Staph. aureus	Pseudomonas sp.	Streptococcus sp.	Klebsiella sp.	Candida albicans
Control	16	18	———	25	_____	_____
Onion	15	21	———	30	_____	_____
Garlic	16	22	———	25	10	11
Arugala	15	17	———	18	_____	_____
Rosemary	17	22	———	32	_____	_____
Thyme	12	17	———	30	_____	_____
Parsley	20	21	———	33	_____	_____
Mint	17	22	———	28	10	10
Cinnamon	17	20	———	33	_____	_____

Table (**3**) Antimicrobial activity of different essential oils on the isolated bacteria vs. trimethoprim-sulfamethoxazole antibiotic.

Bacteria OSE	E. coli	Staph. aureus	Pseudomonas sp.	Streptococcus sp.	Klebsiella sp.	Candida albicans
Control	22	14	_____	_____	20	18
Onion	28	_____	_____	_____	32	25
Garlic	28	25	_____	_____	27	27
Arugala	28	_____	_____	_____	25	25
Rosemary	35	_____	_____	_____	25	30
Thyme	25	_____	_____	_____	30	25
Parsley	26	_____	_____	_____	24	25
Mint	30	_____	_____	_____	28	25
Cinnamon	27	_____	_____	_____	31	26

Table (4) Antimicrobial activity of different essential oils on the isolated bacteria vs. Ampicillin antibiotic.

Bacteria OSE	E. coli	Staph. aureus	Pseudomonas sp.	Streptococcus sp.	Klebsiella sp.	Candida albicans
Control	31	20	_____	_____	_____	_____
Onion	27	13	_____	_____	_____	_____
Garlic	28	14	_____	_____	11	12
Arugala	25	13	_____	_____	_____	_____
Rosemary	28	20	_____	_____	_____	_____
Thyme	29	15	_____	_____	_____	_____
Parsley	30	14	_____	_____	_____	_____
Mint	28	17	_____	_____	_____	_____
Cinnamon	28	15	_____	_____	_____	15

Table (**5**) Antimicrobial activity of different essential oils on the isolated bacteria vs. Norfloxacin antibiotic.

Bacteria OSE	E. coli	Staph. aureus	Pseudomonas sp.	Streptococcus sp.	Klebsiella sp.	Candida albicans
Control	22	23	24	25	28	23
Onion	30	24	28	25	26	28
Garlic	28	26	25	26	26	27
Arugala	24	25	30	30	30	30
Rosemary	30	25	25	24	28	28
Thyme	26	30	32	28	31	33
Parsley	20	23	23	25	30	30
Mint	25	25	26	26	30	28
Cinnamon	22	24	33	26	28	30

Table (6) Antimicrobial activity of different essential oils on the isolated bacteria vs. Amoxicillin antibiotic.

Bacteria OSE	E. coli	Staph. aureus	Pseudomonas sp.	Streptococcus sp.	Klebsiella sp.	Candida albicans
Control	14	15	14	12	23	17
Onion	16	12	21	17	_____	20
Garlic	16	12	11	11	12	22
Arugala	18	13	14	15	13	20
Rosemary	16	12	15	22	_____	20
Thyme	20	12	18	22	12	20
Parsley	17	15	14	25	15	20
Mint	16	12	15	20	10	29
Cinnamon	20	12	10	20	_____	22

Sch (1) Sensitivity test of certain bacteria to essential oils.

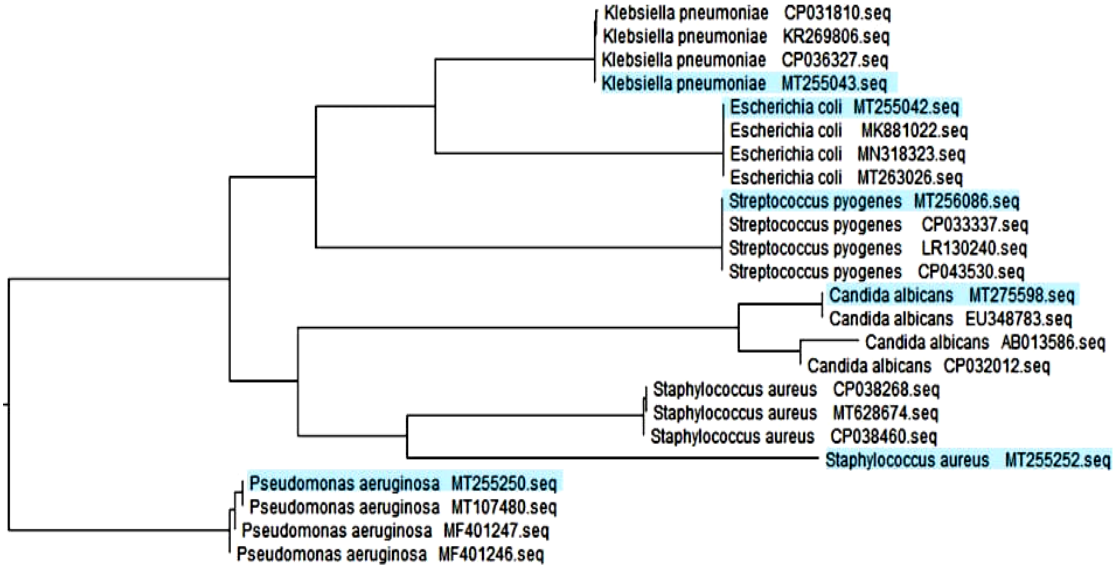

Fig (1) Phylogenetic tree shows the evolutionary relationships between the 16s rRNA sequences of the isolated acne bacteria with their bacterial concatenated nucleotide sequences of their 16s rRNA.

16s rRNA gene sequencin2

In the current study, BLASTn alignments and phylogenetic tree Fig (1) of the assembled 16s rRNA gene sequences showed similarities with the previously partially sequenced 16S rRNA of Candida albicans strain, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes on the NCBI website.

Isolates Accession numbers

Candida albicans strain, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes were deposited in the Gene Bank database and were assigned the accession numbers MT275598, MT255042, MT255043, MT255250, MT255252 and MT256086 respectively .

4. Conclusion and Recommendation

The results of the present study describe the isolation of six different bacteria; Staphylococcus aureus, E. coli, Pseudomonas sp. Streptococcus sp., Klebsiella sp. Candida albicans from Egypt. The antibacterial effects of some medicinal plant oils were tested against the isolated bacteria, Parlesy and garlic oils were found to be rich sources for essential oils with many therapeutic uses. Both are considered good candidates to replace antibiotics in therapy.

5. Discussion

In the present study, we study the Interaction of Some Medical Oils with Antibiotics and Its Effect on Some Pathogenic Microorganisms. Antibiotic resistance is a global problem. Antibiotic resistant bacteria have reached a worrying stage all over the world, mainly in the developing countries [6]. Egypt is one of the countries that have less severe restrictions on antibiotic prescription [8].

It is very common to use essential oils in the treatment of most diseases, for instance skin infectious diseases. Furthermore, it is added as an active constituent in many topical formulations used for the treatment of cutaneous infections for

controlling dandruff, acne, lice, herpes and other skin infections [5].

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