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Antibacterial Effect of Essential Oils Alone and in Combination with Antibiotics against *Escherichia coli* and *Staphylococcus aureus*

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Abstract : *The rapid emergence of multi-drug resistant pathogens has threatened the clinical effectiveness of most of the marketed antimicrobials, which increases the need to find alternatives. Essential oils have been known for their biological activities in the folkloric medicine in many countries for hundreds of years. The aim of the present study was to demonstrate the in vitro antimicrobial property of two commercial essential oils against multi-drug resistant Escherichia coli and Staphylococcus aureus, and to study the effect of combination of essential oils with standard antibiotics. The antimicrobial activity was evaluated by disc diffusion method. The result showed that both tested essential oils have considerably good antimicrobial activity against S. aureus while same cannot be said about E. coli. Grapefruit oil and Neem oil showed no effect on Escherichia coli. The interaction between the tested standard antibiotics and essential oils showed variable results against the tested bacteria. The results are of significance in health care system and microbial disease treatment as the present study showed that essential oils possess good antimicrobial property against tested strains. Most of the essential oil and antibiotic combinations showed synergistic effects. Essential oils can be used as adjuvant to antimicrobial therapy.*

Keywords: *E. coli, S. aureus, essential oils, antimicrobial property.*

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Introduction :

Antibiotic therapy is one of the most important therapies used for fighting infectious diseases and has tremendously enhanced the health aspects of human life since its introduction. Despite the advancements in this therapy, we still live in an era where incidents of antibiotic resistant infections are alarmingly on rise. The significance of the role of antibiotics in nature remains unfounded due to the responses of bacteria through the manifestation of various forms of resistance following the introduction of a new antibiotic for clinical use. The most important factor influencing the emergence and spread of antibiotic resistance is the excessive bacterial exposure to antibiotics. Today, bacteria which are resistant not only to a single drug but simultaneously to many drugs are rampantly spread in the community. (Yap *et al.,* 2014) Nowadays many infections are often caused by multi-resistant microorganisms resulting in difficulty to treat diseases and, consequently, substantial increases in healthcare costs. These multi-resistant microorganisms are reasserting themselves as worldwide threats. The persistence of antibiotic resistance urges the need of finding new therapies against the multi-drug resistant bacteria. (Duin *et al.,* 2016)

Nature is a generous source of compounds with the potential to treat diseases, including infectious diseases. Essential oils produced by plants have been traditionally used for respiratory tract infections, and are used nowadays as ethical medicines for colds. In the medicinal field, inhalation therapy of essential oils has been used to treat acute and chronic bronchitis and acute sinusitis. (Shigeharu *et al*., 2001) Plant essential oils and their major chemical constituents are potential antibacterial agents. Several types of essential oils and their major chemical constituents from various MAPs have been reported to possess a wide range of bacterial inhibitory potentials. The effect of antibacterial activity of essential oils may inhibit the growth of bacteria (bacteriostatic) or destroy bacterial cells. (Swamy *et al.,* 2016)

The widespread non-human use of antimicrobial agents that are regarded as critically or highly important for use in humans creates a reservoir of resistant bacteria and resistance genes that adds to the burden of antimicrobial resistance in human. Humans may obtain antimicrobial-resistant *E. coli* or resistance genes of animal origin directly via contact with animals, food of animal origin, or the environment. These bacteria may subsequently colonize humans or may transfer resistance genes to other bacteria during passage through the intestinal tract. Although the carriage of antimicrobial-resistant *E. coli* in the intestine is not a human health hazard in itself, it may give rise to bacterial infection with limited therapeutic options. (Hammerum *et al.,* 2009) *Staphylococcus aureus* is naturally susceptible to virtually every antibiotic that has ever been developed. This exquisite susceptibility of *S. aureus* led to Alexander Fleming's discovery of penicillin, ushering in the "antibiotic era." Penicillin was truly a miracle drug: uniformly fatal infections could be cured. Yet, by the mid-1940s, only a few years after its introduction into clinical practice, penicillin resistance was encountered in hospitals and within a decade it had become a significant problem in the community. *S. aureus* is remarkable in its ability to acquire resistance to any antibiotic (Chambers *et al.,* 2009).

One major reason why essential oils are powerful against resistant bacteria is because no two batches of an essential oil are exactly the same. The chemical composition of essential oils can differ in different parts of a plant, the stage of plant development, the growth conditions (e.g., temperature, soil and fertilizers), the drying system, and the extraction procedure. The subtle differences in the plant chemistry have been shown to be effective against the adaptation of the *Staphylococcus aureus*, *Escherichia coli* and other bacteria, which is known to adapt itself to make it resistant to many conventional drugs. (Becerril *et al.,* 2012)

Materials and Methods :

Isolation : Escherichia coli isolates were obtained from chicken samples from a local meat market. All samples were placed in a flask containing buffered peptone water and were shaken vigorously. After collection of rinsate, it was inoculated into MacConkey broth and was incubated at 37° C for 24 hours. The color change in the broth was observed. It was then plated onto EMB agar plates and incubated at 37°C for 24-48 hours. After 48 hours of incubation green metallic sheen along with dark colonies were observed on EMB plates (Thanigaivel *et al.,* 2015). *Staphylococcus aureus* was isolated from wound. Wound swab sample was taken from PMCH, Patna. Lawn culture was done on Mannitol Salt Agar plates. After incubation at 37°C for 48 hours the yellow colored circular colonies were observed on MSA plates (Hosimin *et al.,* 2012).

Essential oils : Undiluted commercial essential oils of therapeutic grade were obtained from an online store. The oils used in this study were Grapefruit essential oil (*Citrus paradise*) and Neem essential oil (*Azadirachta indica*)

Antibiotics : The panel of standard antibiotics used to examine the antimicrobial drugs susceptibility-resistance pattern of *Staphylococcus aureus* and *Escherichia coli* isolates were, Chloromphenicol (30mcg), Amoxycillin (20mcg), Ceftazidime (30mcg), Cefuroxime (30mcg), Ampicillin (10mcg), Streptomycin (10mcg), Cefixime (5mcg), and Ceftriaxone (30mcg).

Antimicrobial activity : Antibiotic susceptibility test was performed to detect the susceptibility and resistance of *Staphylococcus aureus* and *Escherichia coli* isolated against the panel of standard antibiotics discs by disc diffusion method (Bauer *et al*. 1966). The effect of essential oil was also tested against the growth of *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. An inoculum of bacterial suspension was prepared and was swabbed over the surface of nutrient agar plate. Sterile paper discs (6mm diameter) were impregnated with 40μl of each commercial essential oil. Plates were incubated for 24h at 37°C. Results of the qualitative screening were recorded as average diameter of the inhibition zone surrounding the discs placed on the agar surface. To evaluate the effect of the combination of the essential oils and antibiotics which were in the form of ready to use discs, 40μl of each essential oil was saturated to the antibiotic disc to determine the zones of inhibition. The obtained results were compared with those of the antibiotics tested on the same strains alone.

Identification of Multi-drug Resistant Bacteria : Strains which are resistant to three or more drugs are considered as multi-drug resistant.

Statistical Analysis : The data recorded during the course of study was analyzed statistically using Chi square test and interpretation was drawn accordingly.

Results and Discussion :

Isolation : Green metallic sheen on EMB agar plates and yellow circular colonies on MSA plates were obtained. Cultural and morphological characteristics were observed and different biochemical tests were performed, confirming that the isolated organisms were *Escherichia coli* and *Staphylococcus aureus* (Table 1). However 16S ribosomal RNA sequencing can further be done for confirmation of bacteria at species level.

Citrus paradise : Grapefruit essential oil showed antibacterial activity against *Staphylococcus aureus* but it was ineffective against *Escherichia coli* (Table 3). Plant J. *et al* (2015) also showed that grapefruit shows little to no activity against the *E. coli*. The essential oil of grapefruit shows significant antibacterial character against *S. aureus.* The application of grapefruit oil with streptomycin, ampicillin, cefixime, ceftriaxone, ceftazidime, chloromphenicol and cefuroxime increased the antimicrobial activity of these tested antibiotics. However an antagonistic effect was seen in some combination of grape fruit essential oil and antibiotics (Table 4).

Azadirachta indica : Neem essential oil had no antibacterial activity against *Escherichia coli*; however it showed average activity against *S. aureus* with an inhibition zone of (6 ±1) mm (Table 3). However, Jahan *et al.* (2007) in her study on effect of neem oil on some pathogenic bacteria found that the antibacterial activity of neem oil showed 92% susceptibility against *Escherichia coli.* The antibiotics, ampicillin, cefixime, chloromphenicol and amoxicillin, showed synergistic effect against *E. coil* in combination with the neem oil, while in the case of *S. aureus* they mostly showed antagonistic effect. The combinations consisting streptomycin and ceftriaxone were the only ones that showed synergy in case of *S. aureus* (Table 5).

Multi drug resistant bacteria : Isolated *E. coli* strain was found resistant to streptomycin, ceftazidime, ampicillin, cefixime, chloramphenicol, cefuroxime and amoxicillin, while *S. aureus* was found resistant to ampicillin, ceftazidime, chloramphenicol and cefuroxime (Table 2). Both the organisms were resistant to more than three drugs. Thus the isolated organisms were multi-drug resistant.

Statistical Analysis : The data analyzed by Chi-Square test showed that there was an association found between the antibacterial effect of essential oil and standard antibiotics.

Conclusion :

This study indicates that the combination of essential oils of two medicinal plants and the standard antibiotics has significant potentials for the development of new antimicrobial treatment and reduction of drug resistance, which will permit to find the treatment of several disease caused by microorganisms. From the data analysis conducted using Chi square test during the course of study, it was found that there is an association between the antimicrobial property of essential oils and standard antibiotics. From the results obtained, the essential oil was found mostly to act in synergy with the tested standard antibiotics. This synergy could lead to new options for the treatment of infectious diseases and emerging drug resistance. There is a need for more studies on the molecular basis of the synergistic interaction to understand the synergistic mechanism that is fundamental for the development of pharmacological agents to treat bacterial infections using medicinal plants. Therefore, research should be focused in that direction to identify medicinal plants that have synergistic behavior.

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Table 1. Result of the biochemical characterization of the isolates

Biochemical test	E. coli	S. aureus
Catalase	+ve	+ve
Coagulase	-ve	+ve
Motility	+ve	-ve
Citrate	-ve	+ve
Gelatin hydrolase	-ve	-ve
Indole	+ve	-ve
MR	+ve	+ve
VP	-ve	+ve
Nitrate reductase	+ve	+ve
Oxidase	-ve	-ve
Urease	-ve	+ve

Table 2. Antibiotic sensitivity test of *E.coli* and *S.aureus*

Antibiotics	Concentration of antibiotics	Zone of resistance (mm)	Zone of susceptibility (mm)	Diameter of zone formation by *E.coli* (mm)	Diameter of zone formation by *S.aureus* (mm)
Streptomycin	10mcg	14	15	R 4	S 16
Ampicillin	10mcg	13	17	R 0	R 10
Ceftriaxone	30mcg	13	21	S 26	S 20
Ceftazidime	30mcg	14	18	R 0	R 0
Cefixime	5mcg	15	19	R 12	S 22
Chloromphenicol	30mcg	11	23	R 8	R 16
Cefuroxime	30mcg	14	23	R 2	R 2
Amoxycillin	30mcg	19	20	R 10	S 30

R = Resistant, S = Susceptible, SS = Slightly susceptible

Table 3. Antimicrobial activity of essential oils on *Escherichia coli* and *Staphylococcus aureus*

Essential oil	Amount of essential oil per 6mm diameter disc	Diameter of zone formation *E. coli* (mm)	Diameter of zone formation *S.aureus* (mm)
Grape fruit oil	40µl	0	4
Neem oil	40µl	0	6

Table 4. Antimicrobial activity of grapefruit essential oil in combination with antibiotics on *Escherichia coli* and *Staphylococcus aureus*

Antibiotics	Concentration of antibiotics	Zone of resistance (mm)	Zone of susceptibility (mm)	Diameter of zone formation *E.coli* (mm)	Diameter of zone formation *S.aureus* (mm)
Streptomycin	10mcg	14	15	R 6	S 20
Ampicillin	10mcg	13	17	R 2	R 2
Ceftriaxone	30mcg	13	21	S 28	S 28
Ceftazidime	30mcg	14	18	R 2	R 2
Cefixime	5mcg	15	19	R 14	R 4
Chloromphenicol	30mcg	11	23	R 8	SS 18
Cefuroxime	30mcg	14	23	R 2	R 12
Amoxycillin	30mcg	19	20	R 2	S 24

R = Resistant, S = Susceptible, SS = Slightly susceptible

Table 5. Antimicrobial activity of neem essential oil in combination with antibiotics on *Escherichia coli* and *Staphylococcus aureus*

Antibiotics	Concentration of antibiotics	Zone of resistance (mm)	Zone of susceptibility (mm)	Diameter of zone formation *E.coli* (mm)	Diameter of zone formation *S.aureus* (mm)
Streptomycin	10mcg	14	15	R 2	S 18
Ampicillin	10mcg	13	17	R 2	R 6
Ceftriaxone	30mcg	13	21	S 24	S 30
Ceftazidime	30mcg	14	18	R 0	R 0
Cefixime	5mcg	15	19	SS 18	R 0
Chloromphenicol	30mcg	11	23	SS 16	R 6
Cefuroxime	30mcg	14	23	R 0	R 0
Amoxycillin	30mcg	19	20	R 14	S 28

R = Resistant, S = Susceptible, SS = Slightly susceptible

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