INVESTIGATION OF ANTIBACTERIAL AND ANTIOXIDANT ESSENTIAL OILS IN NANO EMULSIONS

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DECLARATION

This research project is exclusively my own work and has not been prior presented for the award of any degree in any university for any other award.

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Signature …….. Date…….

SUPERVISOR APPROVAL

I can confirm that this project has been submitted for examination with my approval as university supervisor.

PROFF. BINDU MADHAVI

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Signature…… date…….

DEDICATION

I dedicate this work to my family members, relatives and friends for their enormous support all along my academic journey in Mount Kenya University.

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Special thanks to my supervisor professor Bindu for his guidance in performing this research.

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ABSTRACT

Essential oils extracted from edible, therapeutics and herbal plants have been well recognized as natural antimicrobial and antioxidant additives. As viable antimicrobials, essential oils have been observed to progressively control the food borne microbes and as well maintain the wellbeing of the body from other chronic conditions. Due to the hydrophobic nature of the essential oils it's difficult to achieve high antimicrobial property when mixing in the nourishment based items. This has seen the emergence of the Nano-emulsions based essential oils as the solution to this problem. The aim of this study was to formulate the non-emulsion oil containing oregano oil and tea tree oil and after which evaluate its antibacterial and antioxidant activity. The Nano-emulsion was prepared following the standard procedures and it contained the oil phase, aqueous phase and the surfactant. The antibacterial and antioxidant activity of the essential oil based Nano-emulsions were evaluated by disc diffusion and DPPH free radical scavenging assays respectiverly. *L-ascorbic* and was used as the standard antioxidant while ciprofloxacin was used as the standard antibiotic. The antibacterial activity was evaluated against three bacteria; *Pseudomonas aeruginosa*, *E.coli* and *Staphylococcus aureus*. The pH results for the two essential oils at the two different concentration were 6.925± 0.025 and 7.030± 0.020 for 1% and 2% tea tree oil and 7.855 ± 0.005 and 7.390 ± 0.010 for 1% and 2% oregano oil. The results for the antibacterial activity showed both essential oil at 2 % had significantly higher antibacterial activity ($p<0.05$) against all the bacterial. The Nano-emulsion containing oregano had higher antibacterial activity against *E.coli* while the tree tea oil was more effective against *Psudomonanos aeuruginosa.* The results for the antioxidant activity of the tea tree oil Nano-emulsion was not significantly different from each another at all dose for both 1% and 2% ($p>0.05$). However, L-ascorbic acid recorded higher antioxidant activity by recording lower IC50 concentration of 309.9 followed by 2 % tea tree oil Nano emulsion with 570.7 and lastly 1 % tea tree oil Nano emulsion with 1639.6. In conclusion, the incorporation of the essential oils in Nano-emulsions which is formulated in different ingredients enhances their antimicrobial and antioxidant activity.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Currently, there is increased development of antibacterial agents' resistance. These is as a result of inappropriate use of antimicrobials and since stern measures are yet to be enforced to stop these practices, it necessitates the need to research for compounds that have antimicrobial effect for instance the essential oils in leaves, barks, flowers, roots of many plants and document. These essential oils could be encapsulated in Nano emulsions and could be essential in providing readily available alternative antimicrobials, cheap antimicrobials, less toxic and tolerable antimicrobials this could help in preventing insufficient or lack of antimicrobials in the health sectors. The healthcare givers have no options to manage a given infection resistance especially with decreased rate of antimicrobial drug discovery currently (Pathania et al., 2018).

1.2 problem statement

The current bacterial resistance status is worsening each and every day as many bacteria have become resistant to the available antibiotics (C Reygaert, 2018). This has been triggered by the increased misuse and inappropriate use of the available antibiotics (Buffet-Bataillon et al., 2012). The cost of health care has as well increased due to the more sophisticated equipment facilities being need and more days spend in the hospitals. Herbal management of bacterial infection and oxidative stress related conditions that involves use of essential oils has been of help (Man et al., 2019). However, the difficulties encountered in incorporating the essential oils in other delivery systems has greatly limited its use due to low water solubility and intense aroma (Lucia & Guzmán, 2021).

Hence there is need to employ the Nano-technology that will involves incorporating the essential oils in Nano-emulsions for quick and efficient delivery (Guerra-Rosas et al., 2017).

role in heart disease, cancer and other diseases (Berenice Moreno-Trejo et al., 2019).

1.4 Justification

Essential oils have strong antibacterial activity against many bacteria such as*Escheria coli, pseudomonas aeruginosa* and *staphylococcus aureus* (Solomakos et al., 2008). Incorporation of these essential oil in emulsions enhances their stability and could be administered as unit dosage forms. Additionally, the properties of the essential oils such antibacterial and antioxidant are enhanced by incorporating in Nano-emulsions (Qian & McClements, 2011). By the virtue of it being medically helpful in managing conditions that arise due to resistance of most antibiotics in the market today. Therefore, there is a need to investigate whether these essential oils incorporated in Nano emulsions are active in acting against the resistant microorganisms and this justifies the need to investigate if it exerts both antioxidant and antimicrobial activities that help alleviate the illness

1.5 objectives

1.5.1 General objectives

To investigate antioxidant and antimicrobial activities of essential oils in Nano-emulsions.

1.5.2 Specific objectives

I. To investigate antioxidant properties of essential oils in Nano-emulsion.

II. To investigate the antimicrobial activities of Nano-emulsions.

1.6 research questions

I. Does the essential oils incorporated in Nano-emulsions have antioxidant activity?

II. Does the essential oils incorporated in Nano-emulsions have antimicrobial activity?

CHAPTER: LITERATURE REVIEW

2.1 Bacterial resistance

Bacterial resistance refers to unresponsiveness of a microorganism to an antimicrobial agent or the capacity of a bacteria to withstand the effects of antibiotics that are intended to kill or control them. Antibiotic resistance is one of the biggest threats to global health today. The main causes of antibacterial resistance includes over prescription of antibiotics, patients not finishing the entire antibiotic course, poor infection control in health care settings, absence of new antibiotics being discovered , overuse of antibiotics in livestock farming.

Bacterial resistance against majority of common antibiotic is aided through various mechanisms (Reygaert, 2016). The bacteria gain resistance by interfering with the antibiotic limiting its arrival at the target site. This is aided by enzyme activity such as the beta-lactamase enzyme that breaks the beta-lactam ring in penicillin opening it hence interfering with its structure (C Reygaert, 2018).

In other instances the antibiotic may be modified by addition of an extra group that changes the entire structure of the antibiotic hence interference with its activity. For instance aminoglycosides N acetyl transferases add an additional acetyl group to kanamycin hence stops it from binding to the ribosome (C Reygaert, 2018)

Similarly, the antibiotic target point may be modified making it unrecognizable with the antibiotic. For example the mutation in the penicillin binding proteins this prevents binding of the antibiotic hence the antibiotic with this as the target sites remain ineffective. The antibiotic in other instances may be removed from

the bacteria as toxins before it elicits the indented activity. This is aided by the efflux pumps (C Reygaert, 2018).

2.2 Antibacterial agents

Antibacterial agent are substances that either kill or inhibit the growth of bacteria. These agents are either synthetic or natural in nature where they are produced from other micro-organism, animals or plants. The classification of antibiotics is done based on various criteria such as mode of action, structure and the target organism (Ullah & Ali, 2017).

Based on structure, many antibacterial agents are identified. The classification based on this criteria is very vital as different structures of the antibiotics influence in the different mechanism of action that has been witnessed. Similarly different properties such as toxicity and efficacy are similar in antibiotics of same parent structure. Different groups of antibiotics/ antibacterial agent are identified based on this classification criteria. These include; Sulphonamides such as sulfadiazine, Diaminopyrimidines such as include trimethoprime, Quinolones such as ciprofloxacin, Beta lactam antibiotics such as penicillin's and carbapenemes, Tetracycline's such as chlortetracycline, Aminoglycosides such as gentamicin and Polyene antibiotics such as nystatin and amphotericin B.

On mode of action of the antibacterial or antibiotics two broad categories of antibiotics are identified; bactericidal and bacteriostatic (Aminov, 2010). The bacteriostatic antibiotics/antibacterial agents don't totally prevent the growth of the micro-organism but only inhibit the growth. Upon diminishing of the antibacterial agent, the micro-organism regenerates and grows again. On the other hand the bactericidal antibiotics totally kill the micro-organism and upon

the diminishing of the antibacterial agent the micro-organism cannot regenerate again (Ullah & Ali, 2017). Based on this classification antibiotics may be group based on the mechanism of action they elicit they antibacterial activity. Here the antibiotic may inhibit the cell wall synthesis, cell membrane leakages, and inhibiting of protein synthesis.

The antimicrobial agents can as well be classified based on the target organism. Based on these criteria antimicrobials such as antibiotics that act on bacteria are identified. Further other antimicrobial such as antivirals which act on virus, antifungals that act on fungi, anti-helminths that act on helminths and antiparasitics that control parasites.

2.3 Antioxidant activity

The antioxidant activity is the ability to inhibit the deleterious effects of free radicals such as lipid peroxidation by the antioxidant molecules. The free radicals that may be of both nitrogen and oxygen based are very reactive due to the characteristic presence of unpaired electrons. These radicals are produced under normal physiological process and also under influence of other factors such as smoking of tobacco or exposure to radiations (Valko et al., 2007). These radicals are very important when under normal concentration. However, under increased concentrations they result into imbalance that cause condition known as oxidative stress that is a player in chronic disorders such as cancer. Antioxidants are key in controlling this condition by either inhibiting, terminating the chain reactions or scavenging the excess free radicals (Lourenço et al., 2019).

Natural antioxidants from obtained from plant materials and other natural sources include polyphenols such as flavonoids, anthocyanins, lignans, and

carotenoids, essential oils and vitamins. These antioxidant are very viatla nad have greatl contributed to the reduction of many chronic conditions that are witnessed in many people. On addition these antioxidant sources have been regarded safer and potent as well (Lobo et al., 2010).

2.4 Bacteria

2.4.1 *Pseudomonas aeruginosa*

It's a gram negative and fucultatively aerobic bacterium that can cause disease in plants and animals. Generally it infects immunocompromised but can also affect immunocompetent persons. It's difficult to treat because of its natural resistance to antibiotics (Gellatly & Hancock, 2013).

It's found in soil, water, skin flora and most man made environments throughout the world. Symptoms of infection include inflammation and sepsis. Pseudomonas causes 10-20% infections especially in patients with burn wounds, cystic fibrosis, acute leukaemia, organ transplant and IV drug addiction (Gellatly & Hancock, 2013).

2.4.2 *Staphylococcus aureus*

It's a gram positive bacterium found in the upper respiratory tract and on the skin. These bacteria are spread by having direct contact with an infected person, by using a contaminated object or by inhaling infected droplets dispersed by coughing and sneezing (D.lowy & M.D., 1998).

Staphylococcus infections are caused by staphylococcus bacteria. Staphylococcus aureus causes a wide range of infections. It's the leading cause of blood stream infection, bacteraemia, infective endocarditis, it also causes osteoarticular, skin and soft tissue, pleuropulmonary and device related infections (Rasigade & Vandenesch, 2014).

Strains of bacteria that are resistant to almost all beta lactam antibiotics are called methicillin resistance staphylococcus aureus MRSA. Methicillin is a type of penicillin (Chambers, 2005).

2.4 .3 Escherichia coli

E.coli is a gram negative anaerobic, rod shaped bacteria of the genus Escherichia commonly resides in the intestines of humans and animals (Guo et al., 2017). Most types of E. coli are harmless and help keep your digestive tract healthy. Most commonly contaminated food are ground beef, unpasteurized milk and fresh produce ie spinach and lettuce which are vulnerable to E. coli contaminations. Other types includes *E.hermannii* and *E.vulneris* which are found in human wound infections .*E.fergusonii* is most often isolated from human faeces. *E.blattae* is a commensal organism of cockroaches but is not recovered in human specimens(Guo et al., 2017).

CHAPTER THREE: MATERIAL AND METHODS

3.1 Source of essential oils

The tea tree and oregano essential oils used in this study were purchased from the market. These oils were pure distillates and upon arrival in the laboratory were kept in accordance with the instructions outlined by manufacturer.

3.2 Solvents, reagents, equipment and apparatus

All the solvents and reagents used in this study were all pure and of analytical grade obtained from credible sources. They included methanol, L-ascorbic acid,

2, 2- diphenyl picrylhydrazyl radical (DPPH), tween 80, distilled water, Muller Hilton agar, sodium lauryl sulphate and pure ciprofloxacin discs (30 mcg).

The equipment included, analytical balance, double beam spectrophotometer, hotplate, Ultrasonic homogenizer meter, compound microscopic and autoclave. The apparatus included; test tubes, conical flasks, petri dishes,

3.3 Preparation of the nono emulsions containing essential oils.

The nano-emulsion containing essential oils at the two different concentrations 1% and 2% were prepared according to the method described by with minor modifications. First, 2 % tween 80 solution was prepared in distilled water by dissolving 2ml of tween 80 in 98 ml of distilled water. This was done under room temperature and the mixture stirred on a magnetic stirrer for 10 minutes to obtain a homogenous solution. Into this solution in separate conical flasks the respective essential oil (tea tree oil and oregano oil) were added at different concentrations of 1 % and 2 % each by use of a micropipette. The entire mixture was stirred on magnetic stirrer for 15 minutes followed by sonication on the ultrasonic homogenizer for a period of 20 minutes.

Sample name	Essential oil type	Essential oil% v/v	Tween 80% v/v	SlS% w/v
E/O	*Oregano*	1	2	0.25
E/T	*Tea tree*	1	2	
E/O	*Oregano*	2	2	0.25
E/T	*Tea tree*	2	2	

3.3.1 Characterization of the essential oil nano-emulsion

The properties of the prepared nano-emulsions were determined the stability of the nano emulsion. These properties included, pH, particle morphology and turbidity.

3.3.1.1 pH

The pH of the prepared nano emulsion was measured by the use of pH meter (labman). This involved first calibrating the pH meter with the three standards (10.01, 7.01 and 4.01). After immersion of the pH meter probe in the respective standard it was rinsed with distilled water. After calibration the probe rinsed with distilled water was immersed in the respective Nano-emulsion and the pH values recorded. This was done in duplicate for each Nano-emulsion.

3.3.1.2 Particle morphology

The Nano emulsion droplets morphology was observed under light compound microscope. This involved adding a droplet of the respective Nano-emulsion on a clean slide and observation made at both x 40, x100 and x100 and x400 magnification points.

3.3.1.3 Turbidity

The turbidity of the prepared Nano-emulsions was checked by determining the ability of light to pass through them. This involved measuring the absorbance of the respective essential oil of essential oil Nano emulsions at 600 nm wavelength using a uv-vis spectrophotometer. The measurements were made in triplicates.

Sample name	Essential oil type	Essential oil% v/v	Tween 80% v/v	SlS% w/v
E/O	*Oregano*	1	2	0.25
E/T	*Tea tree*	1	2	
E/O	*Oregano*	2	2	0.25
E/T	*Tea tree*	2	2	

3.4 Antibacterial activity of Nano-emulsion

3.4.1. Test micro-organisms

Three test microorganisms; two gram negative and one gram positive were used in this study. The bacteria included the pure cultures of *E. coli, Staphylococous* and *Psudomonaous aeruginosa* obtained from the microbiology laboratories

located in the centre for infectious diseases in Kenya medical research institute (KEMRI). These bacteria strains were aseptically transported in the biohazard bags to the microbiology laboratory of Mount Kenya University. Here they were sub cultured to check their viability and also obtain fresh colonies.

3.4.2 Preparation of the culture media

The culture media used in this study was the nutrient agar (Hi-media Mumbai) and it was prepared in accordance with the manufacturers' instruction. The instruction directs that 28 grams of the pure powder be suspended in 1000 ml of distilled water. In this study exactly 5.8 grams of the powder was suspended in 200 ml odd distilled water in a 500 ml conical flask and then boiled water on a hotplate to dissolve. The well dissolved media was coved with a foil paper and sterilized by autoclaving for 15 minutes at 15bars pressure and 121^0C.the sterile media was allowed to cool to about 45-50^0C and then plated on sterile petri dishes with each plate carrying 20 ml.

3.4.3 Antimicrobial activity

The antimicrobial activity was evaluated by disc diffusion technique as described by with minor modifications. This briefly included, inoculating the respective bacteria strains on the respective labelled uniformly using a sterile swabs. This involved first preparing the bacteria inoculants whose turbidity was compared to the 0.5 Mac faland standard. A sterile swab was then dipped in the respective bacteria strain inoculant and then spread uniformly on the sterile media. Using a sterile forceps, sterile cylindrical paper disc were laid on the bacteria inoculated media and 20 ul of the respective Nano- emulsion top loaded using a micropipette. Ciprofloxacin disc (30 mcg) were laid at the centre of every plate and all the plates incubated in an incubator for 18 – 24 hours. The

zones of inhibition around the discs were then measured using a ruler and recorded in millimetres.

3.5 Antioxidant activity of tea tree oil Nano-emulsion

The antioxidant activity of tea tree oil Nano-emulsion was evaluated by determining its 2, 2 diphenyl picrylhydrazyl (DPPH) free radical scavenging activity. The Nano –emulsion of tea tree oil at 1 % and 2% and L-ascorbic acid were prepared to obtain six different concentrations 0.01, 0.1, 1, 10, 100 and 1000 ug/ml. into clean test tubes 2.6 ml of the Nano-emulsion/ L-ascorbic acid and 1.4 ml of the 0.3 Mm methanolic DPPH solution was added. The reaction mixtures were mixed completely by swirling and incubated in the dark for 15 minutes. The control was prepared by mixing equal 2.6 ml of methanol and 1.4 ml of DPPH solution. After incubation, the absorbance of the Nano-emulsion and L-ascorbic acid were spectrophotometrically monitored at 517 nm using double beam UV-vis spectrophotometer. The percentage free radical activity was then calculated by the formulae;

$$\% RSA = \frac{Abs.C - Abs.T}{Abs.C} \times 100$$

Where Abs.C is the absorbance of the control (methanol + DPPH), Abs.T is the absorbance of the test (sample/L-ascorbic + DPPH).

3.6 Data management and statistical analysis

All the data were first noted in the laboratory note book. The antioxidant activity data was then calculated to get the percentage radical scavenging activity. All the data was then tabulated in the Microsoft excel spread sheet and then exported into the graph pad prism software for analysis. The descriptive statistic was done and the results presented as means± SEM. The percentage radical scavenging activity and zones of inhibition were further analysed by one-way anova followed by tukeys post hoc test to determine the level of significance

between the means. The level of significance was determined at $p<0.05$. the results were then summarized in tables and graphs.

CHAPTER FOUR; RESULTS AND DISCUSSIONS

4.1 PH and absorbance

The pH values of the prepared Nano-emulsions of tea tree oil and oregano are presented in table 4.1. The pH values of both the Nano-emulsions of the two essential oils were between the neutral brackets. The absorbance of the Nano-emulsion were all lower indicating the stability of the Nano-emulsions.

Table 4. 1 pH and Absorbance of tea tree oil and oregano oil non-emulsion

Essential oils	pH	Absorbance
1% Tea tree Nano emulsion	6.925± 0.025	0.154± 0.0003
2% Tea tree Nano emulsion	7.030± 0.020	0.217± 0.0032
1% oregano Nano emulsion	7.855 ± 0.005	0.612 ± 0.0006
2% oregano Nano emulsion	7.390 ± 0.010	2.961± 0.0004

4.2 Antimicrobial activity of tea tree oil and oregano oil non-emulsion

The results of antimicrobial activity of tea tree oil and oregano oil non-emulsion are presented in table 4.2 and figures 4.1 and 4.2 below. The two respective nano emulsions were tested against three bacteria; *E.col, S. aureus and P. aeruginosa*. Against E.coli Nano emulsion containing oregano oil recorded larger zones of inhibition as compared to the Nano-emulsion containing tea three oil (table 4.2). The oregano oil Nano emulsion at 2 % significantly inhibited the growth of E. coli as compared to the oregano oil Nano emulsion at 1 % ($p<0.05$; fig 4.1). Similarly, the tea tree Nano-emulsion at 2 % recorded significantly larger zone of inhibition as compared to tea tree Nano-emulsion at 1 % ($p<0.05$; fig 4.1). The standard antibiotic (ciprofloxacin 30 mcg) used as the reference significantly recorded the largest zone of inhibition against *E. coli* as compared to the two nano-emulsions ($p>0.05$; fig 4.1).

Against S. aureus the two nano-emulsions recorded no significant difference in the zones of inhibition ($p>0.05$; fig 4.1). However, a significant difference was noted in the zones of inhibition recorded by the nano emulsions containing 1 % and 2 % of the respective essential oils. ($p>0.05$; fig 4.1). Similarly, the standard (ciprofloxacin 30 mcg) recorded significantly larger zone of as compared to the two nano-emulsions ($p<0.05$; fig 4.1).

The Nano emulsion containing tea tree essential oil at both percentages larger zones that were significantly different from the zones of Nano emulsion containing oregano essential oil ($p<0.05$; fig 4.1). However, no significant difference was noted in the zones of inhibition recorded between 1% and 2% of the respective oils in the Nano emulsions ($p>0.05$; fig 4.1). Standard (ciprofloxacin 30 mcg) recorded significantly larger zone of as compared to the two nano-emulsions ($p<0.05$; fig 4.1).

The nano-emulsion containing tea tree oil at 1 % was more active against *P.aeruginosa* that the other two bacteria (*E.coli and S.aureus*). This was evidenced by the significamtly largewr zone of inhibition recorded ($p<0.05$; fig 4.2). The tea tree oil Nano emulsion at 1 % recorded significantly least zone of inhibition against E.coli ($p<0.05$; fig 4.2). The zone of inhibition recorded by nano-emulsion containing tea tree oil at 2 % was not significantly different between *S.aureus and P.aeruginosa* ($p>0.05$; fig 4.2). However, tea tree oil Nano emulsion at 2 % recorded significantly smaller zone of inhibition against E.coli as compared to both S.aureus and P.aeruginosa ($p<0.05$; fig 4.2).

The oregano oil Nano emulsion at both 1 % and 2 % recorded significantly larger zones of inhibition against E.coli as compared to *S.aureus* and

P.aeruginosa ($p<0.05$; fig 4.2). Similarly, significantly smaller zones of inhibition were recorded against *P.aeruginosa.* the standard antibiotic (ciprofloxacin 30 mcg) significantly recorded larger zone of inhibition against E.coli as compared to both S.aureus and P.aeruginosa ($p<0.05$; fig 4.2).

Table 4. 2 antimicrobial activity of tea tree oil and oregano oil non-emulsion

Bacteria	Mean zones of inhibition				
	Tea tree Nano emulsion		Oregano oil Nano emulsion		standard
	1%	2%	1%	2%	30 mcg/ml
Escherichia coli	6.00± 0.000	8.500 ± 0.5	12.000 ± 0.000	14.500± 0.500	42.5 ±0.50
Staphylococcus aureus	8.00 ±0.000	10.500± 0.5	8.250± 0.750	10.500 ± 0.5	34. 5± 0.5
Pseudomonas aeruginosa	10.00± 0.000	11.000± 0.00	6.500± 0.500	7.500± 0.5	40.5± 0.5

Figure 4. 1 Antimicrobial activity of tea tree oil and oregano oil non-emulsion

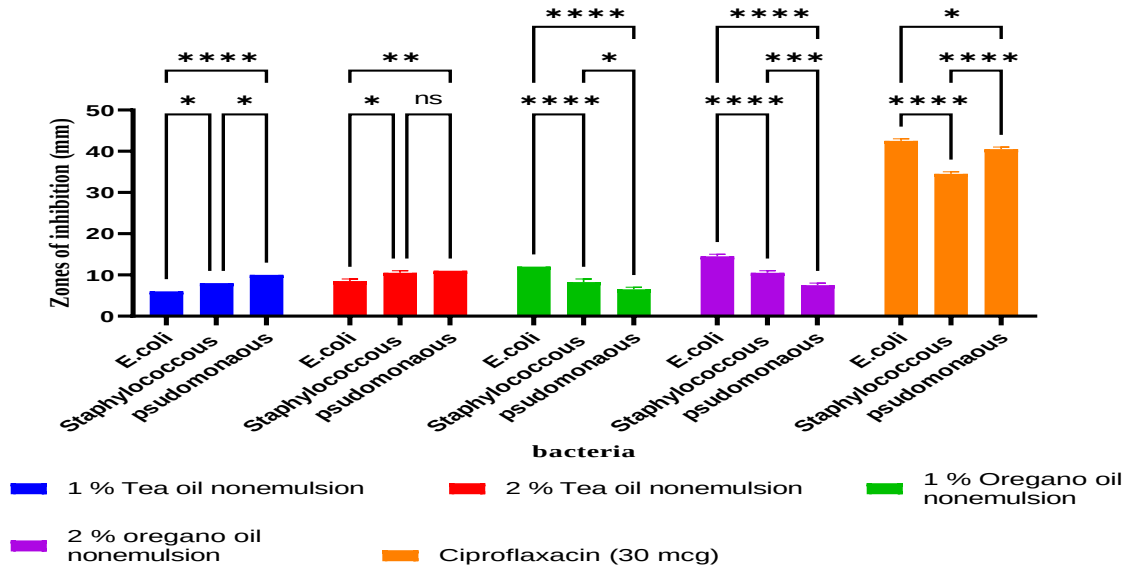
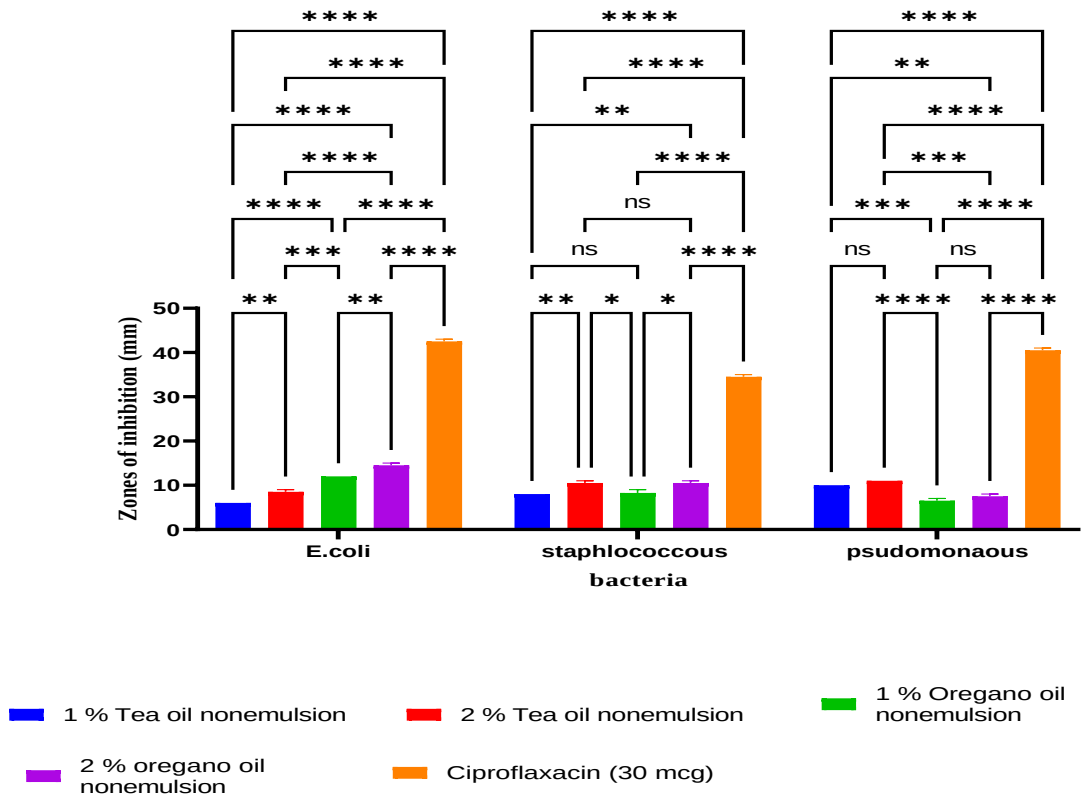


Figure 4. 2 Antimicrobial activity of tea tree oil and oregano oil non-emulsion



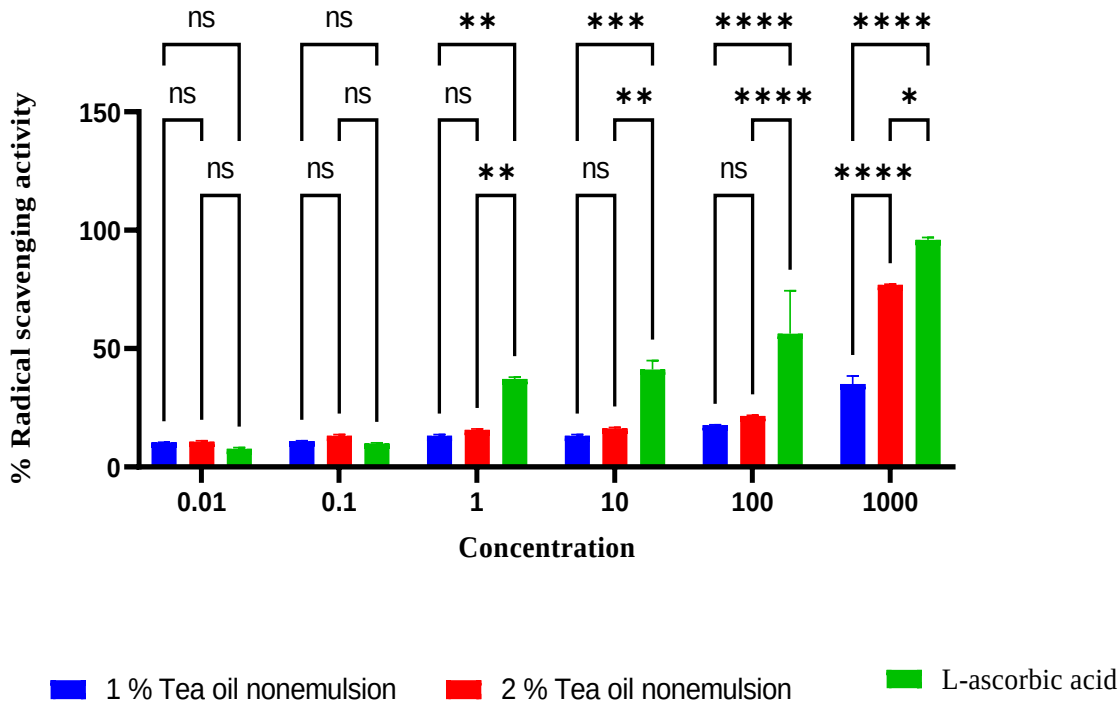
4.3 Antioxidant activity of tea tree oil and oregano oil non-emulsion

The results for antioxidant activity of nano-emulsion containing tea tree ail at both 1% and 2 % are summarized in table 4.3 and figure 4.3. The antioxidant activity was in a dose depended manner with percentage radical scavenging activity increasing with an increase in the concentration. At both 0.01 mcg/ml and 0.1 mcg/ml no significant difference was recorded in the percentage radical scavenging activity for both tea tree at 1 %, 2 % and L-ascorbic acid ($p<0.05$; fig 4.3). at 1,10 , 100 and 100 mcg/ml, L-ascorbic acid significantly recorded higher percentage radical scavenging activity as compared to tea tree oil Nano emulsion at both 1 and 2% ($p<0.05$;fig 4.3). However, at 1, 10 and 100 mcg/ml no significant difference was noted in the percentage radical scavenging activity recorded by tea tree oil Nano emulsion at both 1 % and 2 % ($p<0.05$;fig 4.3). at 1000 mcg/ml tea tree oil nano emulsion at 2 % showed the highest percentage radical scavenging activity as compared to 1% ($p<0.05$;fig 4.3).

Table 4. 3 Antioxidant activity of tea tree oil and oregano oil non-emulsion

Concentration	%RSA mean SEM		
	1% tea tree oil	2% tea tree	L Ascorbic acid
0.01	10.390± 0.081	10.640± 0.462	7.667± 0.555
0.1	10.983 ± 0.130	13.153 ± 0.592	9.953 ± 0.236
1	13.233 ± 0.514	15.733 ± 0.297	37.180± 0.809
10	13.233± 0.514	16.363 ± 0.326	41.167± 3.804
100	17.680 ±0.162	21.623 ± 0.130	56.287± 18.162
1000	35.123 ±3.305	76.880 ± 0.263	95.953± 0.982

Figure 4. 3 Antioxidant activity of tea tree oil and oregano oil non-emulsion



CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Essential oils incorporated in Nano-emulsions showed significant antimicrobial effect especially at the stock concentration's (100mg/ml). The emulsion prepared were stable and had good antioxidant concentrations

5.2 Recommendation

I. To investigate the antimicrobial activity at higher concentrations and to test antioxidant activity of the nano emulsion against a standard

II. The minimum inhibitory concentration oregano essential oil in Nano-emulsion be determined

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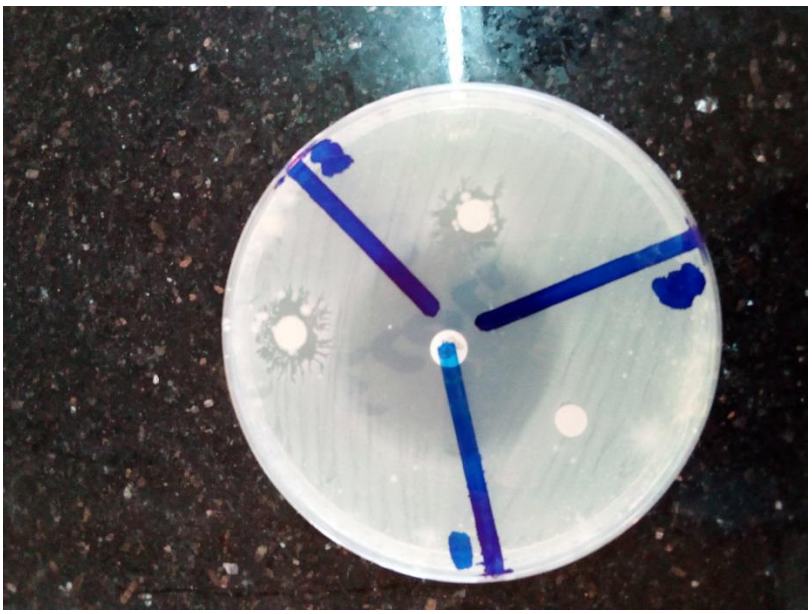
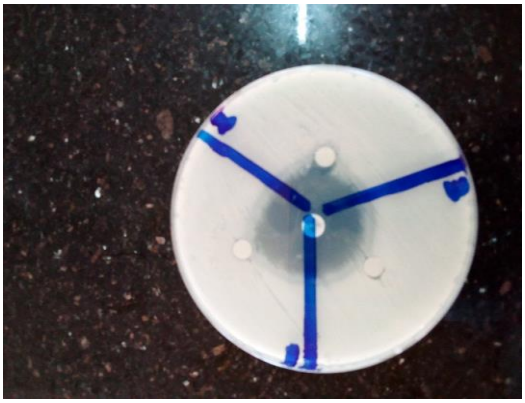
Appendices

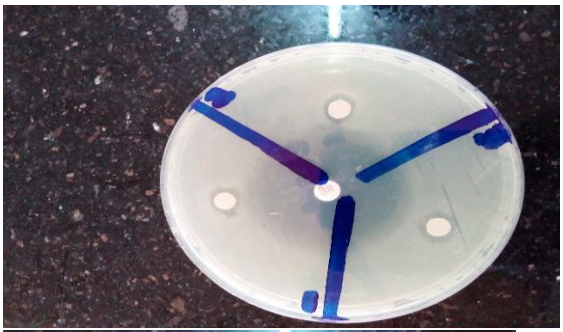
E. coli

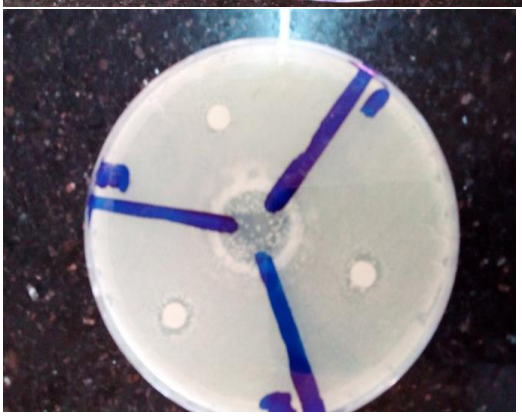


Staphylococcus aureus



Pseudomonas aeruginosa






78-1 MAGNETIC HOT PLATE
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POWER
HEAT
INDICATE


1% v/v
Oregano
POWER