Antimicrobial Activities of *Coriandrum sativum*, *Anethum graveolens* and *Linum usitatissimum* Essential Oil-Nanoemulsions For Use as Alternatives Food Preservative

Alia M. Aldahlawi,[1,2] Ghaida S. Bin Siddik[1] and Magda M. Aly[1,3*]

[1]Department of Biological Science, Faculty of Science, King Abdulaziz University Jeddah, Saudi Arabia

[2]Immunology Unit, King Fahad Medical Research Center, King Abdulaziz University, Jeddah Saudi Arabia,

[3]Botany Department, Faculty of Science, Kafrelsheikh University, Kafr El Sheikh, 33516, Egypt.

ABSTRACT

Bacterial infectious diseases are still one of the main causes of death and severity of bacterial infections, which have markedly gone up mainly due to the emergence of multidrug resistant bacteria. The aim of this study was to prepare nano-emulsions using Coriander and Dill and Flaxseed essential oils and investigate their antibacterial activities. Three nano-emulsions (NEs) were produced by mixing essential oils, surfactants and water with droplet sizes of NEs formulations in the range of 25-62 nm. No toxicity was recorded for Coriander and Dill at 100 µl/ml while Flaxseed NE showed moderate toxicity. Standard local pure cultures of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Candida albicans* were obtained. Dill NEs and Coriander NEs showed moderate activities against both *S. aureus* and *E. coli* with inhibition zone diameter rang ing from 12-14 mm and weak activities against *K, pneumoniae* and *P. aeruginosa*. The three tested Oil Nanoemulsions showed weak inhibition activity with an inhibition zone diameter of 10 mm against *Candida albicans* as a test yeast. The best minimum inhibitory concentrations (MICs) of nanoemulsions was for flaxseed NE against all the tested Gram negative bacteria but the results were higher than that obtained by the control antibiotic that showed excellent activity. In conclusion, the tested NEs showed inhibitory activity against the tested bacteria due to inhibition of vital microbial functions such as cellular transport and/or energy production.

KEY WORDS: PATHOGENIC BACTERIA, NANOEMULSION, ESSENTIAL OILS, DILL, CORIANDER, FLAXSEED.

INTRODUCTION

All over the world, infections with bacteria are considered severe public health problems and almost food-borne diseases are caused by bacteria which cause diarrheal disease, the commonest food borne disease. The Gram-negative bacteria, *Escherichia coli, Shigella* and *Salamonella* in addition to the Gram positive, *Bacillus cereus, Staphylococcus aureus,* methicillin-resistant *S. aureus* (MRSA) were the main causative agents of almost all food-borne diseases. The huge use of antibiotics to treat these dangerous infections led to the emergence of many multidrug resistant bacteria which are the main threats that affect human health (Berglund, 2015; Dadonaite et al., 2018; Awol et al., 2019; Tariq et al., 2019; Al-Nabulsi et al., 2020; Alsayeqh, 2020; Al-Seghayer and Al-Sarraj, 2021).

Mortality and morbidity are increased due to the increase in appearance of multi drug resistant bacteria, thus demand for new antimicrobial agents is increased. There is increasing interest in secondary and aromatic metabolites of medicinal plants due to their well known antimicrobial properties and their success in treatment of several diseases. These plant metabolites can be used especially as natural food preservative instead of chemical ones which were very harmful to human health. Nowadays, several natural materials especially plant essential oils (EOs) have been shown to exhibit broad spectrum inhibitory activities against various Gram positive and Gram-negative pathogenic bacteria and in fact, they can be used to replace synthetic antimicrobial agents. These plant essential oils inhibit undesirable bacterial growth without the appearance of resistant isolates (Dhifi et al., 2016; Tariq, 2019; Long et al., 2019; Raut and Karuppayil, 2014; Long et al., 2019). The antibacterial activities of plants essential oils of oregano, cinnamon, and rosemary, sage thyme, lemon grass, clove, lavender and tea leaves were extensively studied (Najafi-

Article Information:*Corresponding Author: mmmohammad@kau.edu.sa
Received 15/10/2021 Accepted after revision 25/03/2022
Published: 31st March 2022 Pp- 182-187

Available at: https://bbrc.in/ DOI: http://dx.doi.org/10.21786/bbrc/15.1.28

Taher et al., 2018; Gago et al., 2019; Yazgan, 2020; Guo et al., 2020; Falleh et al., 2021).

They showed excellent to moderate antimicrobial activities against both Gram positive and negative bacterial pathogens by denaturation of the bacterial cell membrane which cause potassium and ions leakage in addition to other cell components and finally, cell death. These plant essential oils are hydrophobic compounds and usually have quite low solubility in water, which limits their utilization in aqueous-based foods and beverages. This problem could be simply resolved by encapsulating these essential oils within emulsion-based delivery systems to enhance their biological activities. Therefore, several attempts to incorporate essential oils into different nano-delivery systems have been reported (Donsı and Ferrari, 2016; Tariq, 2019; Aly et al., 2019; Al-Otaibi, 2021; Hassan et al., 2021; Upadhyay et al., 2021; Almasi et al., 2021; Mansouri et al., 2021).

After essential oils are encapsulated into suitable emulsion delivery systems (Nanoemulsion), they can then be incorporated into aqueous-based foods (e.g., beverages) and other products by simple mixing. The nano emulsions has a broad spectrum of activity against bacteria, viruses, fungi and some dermatophytes and spores forming bacteria (Alkhatib et al., 2013, Chime et al., 2014; Chang et al., 2015; Alkhatib et al., 2016 a, b). These nano-emulsion solutions were mainly oil dispersed in water with a diameter ranged from 2-200 nm and stabilized by an interfacial film of surfactant and/or co-surfactant. Essential oil nanoemulsions enhance the antimicrobial properties of EOs and prevent the growth of bacteria. Therefore, the aims of this study were to extract and prepare essential oil nano emulsion from *Coriandrum sativum* (Flaxseed), *Anethum graveolens* (Coriander) and *Linum usitatissimum* (Dill) to be used as alternatives food preservative or as antimicrobial agents against some bacterial pathogens.

MATERIAL AND METHODS

Standard local pure cultures of *Escherichia coli* 25922, *Staphylococcus aureus* 29213, *Pseudomonas aeruginosa* 27853, *Klebsiella pneumoniae* 700603 and Candida albicans 10231 were obtained from Microbiological lab., Faculty of Science, King Abdulaziz University. All the tested isolates were checked for purity using different phenotypic and biochemical assays like Gram reaction, API20E, hemolytic activity and Oxidase, peroxidase and gelatinase tests (Ibrahim et al., 2014). Pre-culture of each test organism was prepared using a nutrient broth medium. Under aseptic conditions, Agar well diffusion method and minimum inhibitory concentration were used to determine the antimicrobial activity of NEs.

Plants seeds were oven dried for 24 hrs at 70°C. Then, the dried seeds were broken into fine powder and essential oils were extracted from 600 g of the dried and powdered seeds by Hexane at 65 - 70°C using Soxhlet extractor (Stony Lab 500 ml Soxhlet Type Extraction Apparatus). The resulting essential oils were stored at -20°C in sealed brown glass vials until use. Water in oil NEs were prepared by mixing 1ml from distilled water, 500 µl from previously extracted essential oils and 100µl from each non-ionic surfactant (tween80, span 20), except for *Linum usitatissimum* where 200 µl from tween80 were used (Alkhatib et al., 2013). The mixtures were sealed and placed in boiling water up to 90-100°C in the water bath until slightly opaque nanoemulsion was formed, similar to the color of their extracted essential oils (Al-Sowayigh et al., 2019, Aldahlawi et al., 2020). Different concentrations of nanoemulsions, 35, 50 and 100 ppm were prepared from each plant oil and all the prepared concentrations were preserved at 4◦C until used.

All prepared NEs were diluted with media (DMEM) and centrifuged at 4500 rpm for 15 min. Then, droplet size and zeta potential of nanoemulsions were obtained at 25°C and analyzed using Zetasizer Nano analyzer. *Artemia salina,* commonly known as brine shrimp, was used as test organism to investigate the toxicity of essential oils nanoemulsions using lethality bioassay technique (Aly and Gumgumjee, 2011; McClements and Rao, 2011; Aldahlawi et al., 2020). About 8 ml of solution containing *Artemia salina* (water life De-capsulated brine shrimp eggs) were added to a hatching chamber containing 300 ml of tap water with a small spoon of food salt and a little of baking soda.

The hatching chamber was left under an inflorescent bulb and air pump for 24-48 h for the eggs to hatch into shrimp larvae. Hatching larvae were collected into a beaker with plastic pipette and separated from the eggs. Then, 10 larvae of *Artemia salina* were collected and added to small Petri plate. The final volume of water in each plate was adjusted to 5 ml with seawater, immediately after adding the larvae. Different concentrations of NEs including 25, 50, 75 and 100 µl were then added to plates and left at room temperature for 4 hrs. After 4 hrs the number of larvae that survived in each plate was determined under stereo microscope (OPTIKA). Percentage of cell mortality of the brine shrimp obtained for each concentration of NEs was recorded.

Under aseptic conditions, Petri plates (90 mm×15 mm) were prepared by pouring 15 ml of sterile Mueller Hinton agar medium in each plate and the agar was allowed to solidify. About 100 µl of freshly prepared inoculum suspensions of bacteria (1.5×10^8 CFU/ml) and yeast ($2x10^6$ CFU/ml) was added using micropipette and uniformly swabbed all over the surface of the Mueller Hinton agar plates using sterile cotton swab by rotating the plate 60 degrees after each application to spread the bacteria and yeast on the surface of the agar plate completely. After inoculum absorption by agar, three wells of 7mm diameter were made in the agar with the help of sterile cork-borer and labeled properly (Nolte and Metchock, 1995). Each well was filled with 100 µl of the nanoemulsion using micropipette. Plates were left for 1-2 hrs at room temperature to allow proper diffusion of the nanoemulsion to occur in the medium. The plates were then incubated without inverting at 37°C for 24 hrs. The average diameter of the inhibition zone surrounding the well was measured (Al-Sowayigh et al., 2019).

Antimicrobial activity of prepared nanoemulsions was determined using Broth microdilution method. In sterile plastic, disposable, microtitration plates with 96 flat-bottom wells, 100 µl/well of sterile distilled water was added into

12 wells using micropipette. The tested microorganisms were prepared from overnight nutrient broth culture and adjusted to 0.5 turbidity using McFarland standard. Then, 5-7 drops of phenol red indicator were added to the adjusted suspensions (Phenol red solution 0.5% in DPBS, purchased from Sigma Chemicals Co., St. Louis, MO, USA). After that, 25 µl/well of prepared bacterial inoculum (1.5×108CFU/ml) and yeast (2x106CFU/ml) was added into 12 wells, and then 125 µl of prepared NEs was added into well number 1 for each organism and mixed properly.

Two-fold dilution of NEs was prepared by transferring 125 µl from well 1 to well 2 and so on and keep diluting and mixing until well 11. The last well 12 serves as growth control (negative control). Finally, the plates were incubated for 24 hrs at 37°C with shaking. The procedure was repeated for each a nanoemulation with all tested organisms. The MIC was determined by changing in the color of the medium. Appearance of pink color indicates the MIC which is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism (Al-Sowayigh et al., 2019). All data were expressed as mean ±standard deviation ($\bar{x}$ ± SD). Statistical analysis was performed with one-way analysis of variance (ANOVA) and pairwise t-test using MegaStat and Microsoft Excel. The statistical significance difference was considered when p-value <0.05.

RESULTS AND DISCUSSION

Zetasizer Nano analyzer was used to identify the Droplet size (Z-average), polydispersity index (PDI), homogeneity or quality of the dispersion and Zeta potential (electrophoretic mobility) of the nanoemulsions formulations. Droplets size was in the range between 25-62 nm. All NEs have negative zeta potential values as shown in Table 1.

Table 1. The Z-average diameters, PDIs and zeta potential measurements of Nanoemulsion formulations expressed as $\bar{x}$ ± SD.

Oil Nanoemulsions		Z-average diameter (nm)	PDI	Zeta potential (mV)
Scientific name	Common name			
Linum usitatissimum	Flaxseed NEs	38.3 ± 8.28	0.216	0.26± 0.048
Coriandrum sativum	Coriander NEs	55.25 ± 6.13	0.111	-2.36 ± 2.74
Anethum graveolens	Dill NEs	28.67 ± 3.13	0.109	-2.17 ± 2.13

Table 2. Toxicity of NEs against *Artemia salina*

Tested NEs	Toxicity (% of mortality)			
	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ml
Flaxseed NEs	20%	33%	30%	50%*
Coriander NEs	10%	23%	20%	20%
Dill NEs	5%	20%	20%	40%
Control	0%	0%	0%	0%

*Toxic Concentration

Artemia salina was used to test the toxicity of 3 different essential oils nanoemulsions. The mortality percentage was determined under stereo microscope. The NE concentration that kills 50% of the brine shrimps (LC_{50}) considered toxic. No toxicity was found for each of the NEs concentrations even for the highest concentration 100 µl, except for flaxseed NE at 100 µl which showed inhibition of 50% of the brine shrimps as shown in Table 2. LC_{50} of flaxseed NEs was obtained at 100 µl/ml. This is meaning that increase NEs concentration may be lead to more mortality percentage of *Artemia salina* larva.

The activity of NEs was tested against pathogenic bacteria and yeast using agar well diffusion method. The results of Table 3 represent the mean diameters of inhibition zones (mm) exhibited by NEs against the tested bacteria and yeast. NEs showed moderate activity against the tested bacteria and *Candida*. Flaxseed NEs showed excellent activity against Gram negative bacteria with mean diameter of 18 mm while low activity was recorded against Gram positive bacteria with mean diameter of 14 mm. Dill NEs and Coriander NEs showed moderate activities against both S. aureus and *E. coli* with inhibition zone diameter ranged from 12-14 mm and weak activities against *K, pneumoniae* and *P. aeruginosa*.

The three tested Oil Nanoemulsions showed weak inhibition activity with inhibition zone diameter of 10 mm against *Candida albicans* as a test yeast. Also, minimum inhibitory concentrations (MICs) of nanoemulsions were determined for the tested pathogens represented in Table 4. The lowest MIC range (19-25 %) was recorded for Flaxseed NEs while the range was 29.7 to 31.0% for Coriander NEs and was ranged from 24.2-42.1 % for Dill NEs. Cefaclor (control antibacterial agent) had MIC ranged from 2-5 µg/ml while Fluconazole (control antifungal agent) had MIC (5 µg/ml) against *Candida albicans*.

A lot of attention was given to nanoemulsions which were produced for protecting and delivering certain plant essential oils. These nanoemulsions were characterized mainly by measuring the droplet size, viscosity, thermal stability and refractive index. Three nanoemulsions (NEs) of Flaxseed, Coriander and Dill were produced by mixing the tested essential oil, surfactants, and water and heating the competent in a water bath until clearance. The physical properties of the obtained NEs formulations were determined and the results were similar to that obtained by

Ozogula et al. (2020) for thyme essential oil nanoemulsions. The roles of the previous nanoemulsions in controlling Dendritic cells phenotype expression, apoptosis, and cytokine secretion were recorded before by Aldahlawi et al (2020). Non-significant effect on the viability and apoptosis of dendritic cells was noticed by these NEs and they have a tolerogenic effect on these cells. The prepared Flaxseed, Coriander and Dill NEs had droplet sizes in the range of 25-62 nm. Similarly, Neem nanoemulsion was successfully prepared and had means droplet size of 67.85 nm (Ghotbi et al 2014; Donsı and Ferrari, 2016).

Table 3. Antimicrobial activity of NEs using agar well diffusion assay.

Tested NEs	Diameter of inhibition zone (mm) mean ±SD				
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
Flaxseed NEs	14.7±0.6ac	18.3±1.5d	18.3±2.1d	18.0±1.0d	10.0±1.0e
Coriander NEs	$13.0\pm1.5_{ac}$	$14.0\pm1.5_{a}$	$11.0\pm1.1_{c}$	$11.0\pm1.4_{c}$	$10.5\pm1.5_{e}$
Dill NEs	$13.0\pm1.0_{ac}$	$14.0\pm0.9_{c}$	$10.0\pm0.5_{c}$	$10.0\pm1.6_{c}$	$10.4\pm2.5_{e}$
Cefaclor*	$25.0\pm1.6_{ac}$	$34.6\pm1.5_{b}$	$39.3\pm1.5_{c}$	$30.0\pm1.0_{b}$	NA
Fluconazole*	NA	NA	NA	NA	$22.0\pm1.3_{f}$

*Cefaclor was used as control for bacteria, Fluconazole was used as control for yeast. NA: Not applicable. The data are presented as mean ± SD, n= 3. The same letters indicated non significant results at $p< 0.05$.

Table 4. MICs of NEs against the tested microorganisms.

Tested NEs	MICs of NEs (%, v/v)				
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*
Flaxseed NEs	25.7±5.0#	21.8±2.9#	21.8±2.2#	19.9±1.9#	43.7±2.0#
Coriander NEs	29.7±2.3#	31.0±3.9#	31.0±4.5	31.0±0.9#	31.0±9.9#
Dill NEs	26.7±1.0#	24.3±2.7#	42.1±11.9	31.0±2.1#	34.3±7.9#
Cefaclor* (µg/ml)	5.0±0.12	2.0±0.09	3.9±0.06	3.9±0.11	NA
Fluconazole* (µg/ml)	NA	NA	NA	NA	5.0±0.03

*Cefaclor was used as control for bacteria, Fluconazole was used as control for yeast. NA: Not applicable., The data are presented as mean ± SD, n= 3. The symbol # : indicates significant results at $p< 0.05$ compared to control.

It was reported that using a mixture of surfactants to prepare nanoemulsions from plant essential oils provides better effectiveness than one. The droplet size increases as the amount of water increases and decreases with the amount of surfactant due to the increase in interfacial area and the decrease in the interfacial tension. It was clear that the amounts of components used in the preparation of NEs affects the droplet size of the nanoparticles. Non-ionic surfactants have hydrophilic and lipophilic molecules that balance size and strength of these opposing molecules is called a hydrophilic-lipophilic balance number (Porras et al., 2004; Ibrahim et al., 2015).

Artemia salina, known as brine shrimp, is one of the standard organisms for testing the toxicity of chemicals. It is the most convenient test organism for toxicity tests, because of its easy hatching from dry cysts and its eggs can be stored for years without losing their viability (Sorgeloos et al., 1978, Ruebhart et al., 2008). All the tested NEs had different antimicrobial activities against all the tested microorganisms, Gram positive and negative bacteria and yeast. The best MIC was for flaxseed NE against all the tested microorganisms. Agar diffusion tests are often used as qualitative methods to determine whether a bacterium is resistant, intermediately resistant or susceptible to the tested antimicrobial agent.

Another method to determine the antimicrobial activity of NEs was minimum inhibitory concentrations (MIC) which is a quantitative method and considered as the 'gold standard' for determining the susceptibility of organisms to antimicrobials, therefore it was used to judge the performance of all other methods of susceptibility testing (Andrews, 2001). Although, the Gram negative

bacteria contain a high lipid layer which is less susceptible to antimicrobial agents, the NE prepared using the surfactant can overcome the lipid barriers and increase the susceptibility of the bacterial cells (Gupta et al., 2014). Ziani et al. (2011) studied the activity of thyme oil (TO) nanoemulsions against some yeast isolates such as *Saccharomyces cerevisiae Brettanomyces naardenensis* and *B. bruxellensis* while Meral et al., (2019) recorded the antimicrobial activities some bacteria, *Pseudomonas aeruginosa*, *E. coli* and *Salmonella typhimurium*.

Nanoemulsions based on essential oil of thyme showed inhibitory activity against some food borne pathogens bacteria (*Salmonella paratyphi, Staphylococcus aureus, Enterococcus faecalis* and *Klebsiella pneumoniae* in addition to some spoilage bacteria of fish. The nanoemulsions affect the cell wall morphology in addition to the damage in bacterial cell membranes. Thus, preparation of nanoemulsions from essential plant oil increased antibacterial activity and can be used as food preservative agent during processing or packaged fish or food products.

CONCLUSION

As a conclusion from experiments that performed on different pathogens, all nanoemulsions had no toxic effect (LC50) at different concentrations when tested against brine shrimp, except for flaxseed NEs that showed the highest inhibition zones against all the tested microorganisms, and the best activity was against Gram negative bacteria, *E. coli*, *K. pneumoniae, P. aeruginosa*. Dill NEs and Coriander NEs showed the highest inhibition against *E. coli*. The best (lowest) MIC was recorded for flaxseed NE against the three tested Gram negative bacterial while higher MICs (lower activities were recorded for coriander NE and Dill NEs. So, all NEs have antimicrobial activity against multidrug resistant pathogens, thus they can be used in medicine and food industries.

ACKNOWLEDGEMENTS

The author would like to thank King Abdulaziz University, King Fahd center for medical research and King Abdulaziz city for Science and technology for the financial grants.

Conflict of Interest: The authors declare no conflict of interests.

Data Availability Statement: The database generated and /or analysed during the current study are not publicly available due to privacy, but are available from the corresponding author on reasonable request.

REFERENCES

Aldahlawi, A. M., Bin Siddik, G. S. and Aly, M. M. (2020). The Efficacy of *Coriandrum sativum, Anethum graveolens*, and *Linum usitatissimum* Essential Oil Nanoemulsions On Human Dendritic Cells. Int.J. Pharm. Res. Allied Sci., 9(4):125-132.

Alkhatib, M. H., Aly, M. M., Saleh, O. A et al. (2016a). Antibacterial activity of a microemulsion loaded with cephalosporin. Biologia 71/7: 1-10.

Alkhatib, M., Aly, M.M. and Bagabas, S. (2013). Antibacterial Activity and Mechanism of Action of Lipid Nanoemulsions against *Staphylococcus aureus*. Journal of Pure and Applied Microbiology 259-267.

Alkhatib, M.H., Aly, M.M., Rahbeni, R. A., Balamash, K. S. (2016b) Antimicrobial Activity of Biocompatible Microemulsions Against *Aspergillus niger* and Herpes Simplex Virus Type 2. Jundishapur Journal of Microbiology, 9(9):e3743.

Almasi, L.; Radi, M.; Amiri, S.; Torri, L. (2021). Fully Dilutable *Thymus vulgaris* Essential Oil:Acetic or Propionic Acid Microemulsions Are Potent Fruit Disinfecting Solutions. Food Chem., 343, 128411.

Al-Nabulsi, A. A., Osaili, T. M., Abu Naser, R. A. et al. (2020). Factors affecting the viability of *Staphylococcus aureus* and production of enterotoxin during processing and storage of White-Brined Cheese. J. Dairy Sci., 103, 6869–6881.

Al-Otaibi, W.A. and AlMotwaa, S.M. (2021). Preparation, characterization, optimization, and antibacterial evaluation of nano-emulsion incorporating essential oil extracted from Teucrium polium L, Journal of Dispersion Science and Technology, 18, 1181–1190.

Al-Otaibi, W. (2021). Rosemary Oil Nano-Emulsion potentiates the apoptotic effect of Mitomycin C on Cancer Cells *in vitro*. PHAR, 68, 201–209.

Alsayeqh, A. F. (2020). Salmonellosis in Saudi Arabia; an Under estimated Disease? Alexandria J. Vet. Sci., 67, 30–38.

Al-Seghayer, M.S. and Al-Sarraj, F.M. (2021). The Outbreak of Food borne Disease by Pathogenic Enterobacteriaceae Antimicrobial Resistance, A Review. AFSJ., 20, 91–99.

Al-Sowayigh, M., Aly, M. M. and Abounasef, S. (2019). Inhibitory effects of novel formula of oil in water Nanoemulsions on some pathogenic bacteria associated with wound infections. IOSR Journal Of Pharmacy And Biological Sciences Volume 14(5):51-57.

Aly, M. M. and Gumgumjee N.M. (2011). Antimicrobial efficacy of *Rheum palmatum, Curcuma longa* and *Alpinia officinarum* extracts against some pathogenic microorganisms. Africian J. Biotechnology, Vol. 10 (56), pp. 12058-12063.

Aly, M.M., Al-Sowayigh, M., Abounasef, S. (2019). *In vitro* Inhibitory Effects of Different Formula of Essential Oils against Bacterial Pathogens Associated with Burn and Wound Infections. IOSR Journal Of Pharmacy and Biological Sciences, Volume 14(3): 70-78.

Andrews, J.M.(2001). Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy,. 48(suppl-1): p. 5-16.

Awol, N., Nigusse, D. and Ali, M. (2019). Prevalence

and antimicrobial susceptibility profile of *Salmonella* and *Shigella* among Food Handlers Working in Food Establishment at Hawassa City, Southern Ethiopia. BMC Res., Notes, 12, 712.

Berglund, B. (2015). Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infect. Ecol. Epidemiol., 5, 28564.

Chang, Y., McLandsborough,L. and McClements, D.J. (2015). Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: essential oil (thyme oil) and cationic surfactant (lauric arginate). Food chemistry, 172: p. 298-304.

Chime, S., F. Kenechukwu, and A. Attama (2014). Nanoemulsions—advances in formulation, characterization and applications in drug delivery, Vol. 3: 2019-2029.

Dadonaite, B., Ritchie, H., Roser, M. (2018). Diarrheal Diseases. Our World in Data, 11-19.

Dhifi, W., Bellili, S., Jazi, S.; Bahloul, N., Mnif, W. (2016). Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review Medicines, 3, 25.

Donsı, F. and Ferrari, G. (2016). Essential Oil Nanoemulsions as Antimicrobial Agents in Food. J. Biotechnol., 233, 106–120.

Falleh, H.; Jemaa, M. B.; Neves, M. A et al. (2021). Formulation, Physicochemical Characterization, and Anti-E. coli Activity of Food-Grade Nanoemulsions Incorporating Clove, Cinnamon, and Lavender Essential Oils. Food Chem., 359, 129963.

Gago, C., Artiga-Artigas, M., Antunes, M. et al. (2019). Effectiveness of Nanoemulsions of Clove and Lemongrass Essential Oils and Their Major Components against *Escherichia coli* and *Botrytis cinerea*. J. Food Sci. Technol., 56, 2721-2736.

Ghotbi, R.S., M. Khatibzadeh, and S. Kordbacheh. (2014). Preparation of neem seed oil nanoemulsion. in Proceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications.

Guo, M., Zhang, L., He, Q., Arabi, S. A. et al. (2020). Synergistic Antibacterial Effects of Ultrasound and Thyme Essential Oils Nanoemulsion against *Escherichia coli*

Gupta, S., Bansal R, Ali J, Gabrani, R et al. (2014). Development and characterization of polyphenon 60 and caffeine microemulsion for enhanced antibacterial activity. BioMed research international,vol. 2014, Article ID 932017, 7 pages,

Hassan, M. E., Hassan, R. R., Diab, K. A et al. (2021). Nanoencapsulation of Thyme Essential Oil: A New Avenue to Enhance Its Protective Role against Oxidative Stress and Cytotoxicity of Zinc Oxide Nanoparticles in Rats. Environ. Sci. Pollut. Res., 28, 1–18.

Ibrahim, I., Al-Shwaikh, R. and Ismaeil, M. (2014). Virulence and antimicrobial resistance of *Escherichia coli* isolated from Tigris River and children diarrhea. Infect Drug Resist.,7:317-322.

Ibrahim, N., Raman, I. and Yusop M.R. (2015), Effects of functional group of non-ionic surfactants on the stability of emulsion. Malaysian J Anal Sci., 19: p. 261-267.

Long, N.; Tang, H.; Sun, F.; Lin, L.; Dai, M. (2019). Effect and Mechanism of Citral against Methicillin-Resistant *Staphylococcus aureus* in Vivo. J. Sci. Food Agric., 99, 4423–4429.

Mansouri, S.; Pajohi-Alamoti, M.; Aghajani, N. et al. (2021). Stability and Antibacterial Activity of *Thymus daenensis* L. Essential Oil Nanoemulsion in Mayonnaise. J. Sci. Food Agric., 101, 3880–3888.

McClements, D. J. and Rao, J. (2011). Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, erformance, Biological Fate, and Potential Toxicity. Crit. Rev. Food Sci. Nutr., 51, 285–330.

Meral, R., Ceylan, Z. and Kose, S. (2019). Limitation of microbial spoilage of rainbow trout fillets using characterized thyme oil antibacterial nanoemulsions. Journal of Food Safetye, 12644.

Najafi-Taher, R., Ghaemi, B.; Kharrazi, S. et al. (2018). A promising antibacterial effects of silver nanoparticle-loaded tea tree oil nanoemulsion: A Synergistic Combination against Resistance Threat. AAPS Pharm. SciTech, 19, 1133–1140.

Nolte, F. S. and Metchock, B. (1995). Mycobacteria. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, & R. H. Yolken (Eds.). Manual of clinical microbiology (pp. 400– 437pp). (6th ed.). Washington, DC, USA: American Society for Microbiology.

Ozogula, Y., Boğaa E. K., Akyolb, I. (2020). Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. Food Bioscience, Volume 36, 100635.

Porras, M., Solans, C., González, C. (2004). Studies of formation of W/O nano-emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 249(1-3): p. 115-118.

Raut, J.S. and Karuppayil S.M. (2014). A status review on the medicinal properties of essential oils. Industrial crops and products, 62: p. 250-264.

Ruebhart, D.R., Cock, I.E. and Shaw G.R. (2008). Brine shrimp bioassay: importance of correct taxonomic identification of *Artemia* (Anostraca) species. Environmental Toxicology: An International Journal,. 23(4): p. 555-560.

Sorgeloos, P., Remiche-Van Der Wielen,C., and Persoone, G. (1978). The use of *Artemia nauplii* for toxicity tests, a critical analysis. Ecotoxicology and environmental safety,. 2(3-4): p. 249-255.

Tariq, S., Wani, S., Rasool, W., Shafi, K. et al. (2019). A

Comprehensive Review of the Antibacterial, Antifungal and Antiviral Potential of Essential Oils and Their Chemical Constituents against Drug-Resistant Microbial Pathogens. Microb. Pathog., 134, 103580.

Upadhyay, N., Singh, V. K., Dwivedy, A. K. et al (2021). Assessment of Nano-encapsulated *Cananga odorata* Essential Oil in Chitosan Nanopolymer as a Green Approach to Boost the Antifungal, Antioxidant and in Situ Efficacy. Int. J. Biol. Macromol., 171, 480–490.

Yazgan, H. (2020). Investigation of Antimicrobial Properties of Sage Essential Oil and Its Nanoemulsion as Antimicrobial Agent. LWT, 130, 109669.

Ziani, K., Chang, Y., McLandsborough, L. et al. (2011). Influence of surfactant charge on antimicrobial efficacy of surfactant-stabilized thyme oil nanoemulsions. Journal of Agricultural and Food Chemistry, 59(11), 6247–6255.