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Essential Oil Nanoemulsion Formulations, Characterization, and Study of its Antibacterial Activity Against Clinical Isolates of *S. aureus*

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Abstract:
From past few years bacterial resistance to antibiotics is posing the severe issue to healthcare sector. Therefore there is urge to find out natural antimicrobial agents. Essential oils are one of the promising option to deal with antibiotic resistance. But poor solubility, stability and odor makes its use restricted. These limitations can be avoided by using nanoemulsion formulations of essential oils. In present study, nanoemulsion formulations of Tea tree oil, Thyme oil, and Clove oil and Cinnamon oil were synthesized using probe ultrasonicator. Physicochemical characterization and stability studies were carried out and tested for droplet size and polydispersibilty index (pdi).Formulated nanoemulsions were tested against clinical isolates of *S.aureus*. The nanoemulsion formulations of Cinnamon oil demonstrated promising results.

Key Words: Antibiotic resistance, Essential oils, Nanoemulsions

1. Introduction:

The use and search of novel drugs and antimicrobial agents derived from plants have accelerated in recent years because of horrific incidences of antibiotic resistance occurring across the globe, even after so much advances made in development of chemical chemotherapeutic agents. Therefore, ethano pharmacologists, botanists, microbiologists, and natural-products chemists are in search of novel phytochemicals based formulations which can be effectively used as potent antimicrobial agents in variety of products for treatment of infectious diseases for reducing the chances of spread of infections. Research in area of plant origin products captured the attention for its application as natural antimicrobial agent in the field of food, drugs and cosmetics. Essential oils derived from plants are amongst one of such preferred agents. Essential oils derived from plants are reservoir of numerous antimicrobial phytochemicals such as carvacrol, eugenol, thymol, cinnamaldehyde, terpineol etc. (Bilia et al., 2014). Significant antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal and insecticidal activities have been demonstrated by essential oils (Kaloustian et al,. 2008; Benjilali et al., 1986; Burt 2004).Therefore essential oil is considered as powerful antimicrobial agent to combat with infections microorganisms. (Stefanakis., 2013).

Mostly essential oils from plants are widely used in agriculture, pharmaceutical, sanitary, cosmetics, and food industries because of their potent antimicrobial bioactive components , but still they pose some disadvantages- like, poor solubility in aqueous medium, low stability, strong aroma and taste, strong organoleptic properties, low stability, volatile nature and limited availability of routes of administration (Huang et al., 2010). This limitation can be overcome by mixing essential oil with surfactant and formulating nanoemulsions (Quian et al., 2012). Antimicrobial nanoemulsions are O/W nanoemulsions with droplet size in range from 200-600 nm and are stabilized by surfactants and demonstrate broad spectrum of activity against variety of microbes including their spores and viruses. Charge interaction between small oil droplets in nanoemulsion and microbial membrane is the reason for the interaction with microbes (www.nanobio.com). Nanoemulsions damage microbes by fusing with lipid bilayers of cell membrane, by releasing energy stored in oil-and- emulsion destabilizing the lipid membrane of the bacteria (Hamouda et al., 2000). The nonspecific action of nanoemulsions, unlike that of antibiotics, thus renders its broad-spectrum effectivity while limiting the generation of resistance. These characteristics make nanoemulsion an appropriate option for both wound treatment (Hemmila *et al.*, 2010) and surface decontamination (Ioannou *et al*., 2007). In present study nanoemulsions of Tea Tree (*M. alternifolia*) oil, Thyme (*T. vulgaris*) oil, Clove(*S.aromaticum*), Cinnamon *(C. zelyanicum)* were synthesized, characterized and tested for its antibacterial activity against clinical isolates of *S.aureus.*

2. Materials and Methods:

2.1 In the present study - Cinnamon oil (*Cinnamomum zeylanicum*), Tea Tree oil (*Melaleuca alternifolia*), Clove oil (*Syzygium aromatic*) and pure Thyme (*Thymus vulgaris*) oil and surfactants, Tween 20, Tween 80 (s-d fine chemicals), deionized water were procured commercially. Hi-sensitivity Test Agar, Nutrient Agar, and Nutrient Broth, from Hi-media laboratories were used. Clinical isolates of *S.aureus* were procured from local Pathology laboratories in Nagpur. Whereas standard reference bacterial culture- *S.aureus* (ATCC 6538) were procured from NCIM_NCL, Pune.

2.2 Nanoemulsion Formulations:

Oil in water nanoemulsions (O/W) of different test essential oils were formulated using different surfactants - Tween 20 and Tween 80. Initially coarse emulsions were prepared with drop wise addition of deionized water to mixture comprising Essential Oil(EO) and Surfactant in ratio of 1:1,1:2,1:3, and 1:4 respectively with simultaneous stirring on magnetic stirrer at 400- 600 rpm for approx.70 minutes. Each crude emulsion was then subjected to ultrasonic emulsification using probe ultrasonicator of 120 watts power at 30 KHz frequency and probe diameter of 15mm. The sonicator was set at cycle of 15 seconds. Each crude emulsion was subjected to ultrasonication for 70 minutes. Sonicator probes generate disruptive forces that reduce the droplet diameter and converts crude emulsion into nanoemulsion. These formulated emulsions were then used for further study.

E.O.	N. E. code	Surfactant Used	Oil: surfactant ratio	% Composition of Different components in Formulations		
				EO(ml)	Surfactant(ml)	Deionized Water(ml)
Tea Tree oil	20T1	Tween 20	1:1	3	3	46
	20T2	Tween 20	1:2	3	6	41
	20T3	Tween 20	1:3	3	9	38
	20T4	Tween 20	1:4	3	12	35
	80T1	Tween 80	1:1	3	3	46
	80T2	Tween 80	1:2	3	6	41
	80T3	Tween 80	1:3	3	9	38
	80 T4	Tween 80	1:4	3	12	35
Thyme oil	20Th1	Tween 20	1:1	3	3	46
	20Th2	Tween 20	1:2	3	6	41

	20Th3	Tween 20	1:3	3	9	38
	20Th4	Tween 20	1:4	3	12	35
	80Th1	Tween 80	1:1	3	3	46
	80Th2	Tween 80	1:2	3	6	41
	80Th3	Tween 80	1:3	3	9	38
	80 Th4	Tween 80	1:4	3	12	35
Clove oil	20 Cl1	Tween 20	1:1	3	3	46
	20 Cl2	Tween 20	1:2	3	6	41
	20 Cl3	Tween 20	1:3	3	9	38
	20 Cl4	Tween 20	1:4	3	12	35
	80Cl1	Tween 80	1:1	3	3	46
	80Cl2	Tween 80	1:2	3	6	41
	80Cl3	Tween 80	1:3	3	9	38
	80Cl4	Tween 80	1:4	3	12	35
Cinnamon oil	20 C1	Tween 20	1:1	3	3	46
	20 C2	Tween 20	1:2	3	6	41
	20 C3	Tween 20	1:3	3	9	38
	20 C4	Tween 20	1:4	3	12	35
	80C1	Tween 80	1:1	3	3	46
	80C2	Tween 80	1:2	3	6	41
	80C3	Tween 80	1:3	3	9	38
	80C4	Tween 80	1:4	3	12	35

Table I: Composition of Essential Oil Nanoemulsions formulated using Tween 20 & Tween 80 as surfactants

2.3: **Physicochemical Characterization of Nanoemulsion Formulations**:
The pH of formulated essential oil nanoemulsions were tested using pH meter (Systronics-361), Visual appearance and %Transmittance was checked with UV-Visible spectrophotometer (Equiptronics) at 600nm. All the parameters were analyzed in Triplicates.

2.4 Stability of Nanoemulsions: Stability studies of prepared nanoemulsions were tested using Heating cooling cycle and thermodynamic stability studies.

2.4.1 Heating –Cooling Cycle: Each of nanoemulsion was kept at 40ºC and 4ºC alternatively for around 48 hrs. This parameter is used to study stability of different Essential Nanoemulsion formulations under study at different temperatures.

2.4.2 Thermodynamic Stability: To prove thermodynamic stability all the formulated nanoemulsions were centrifuged at 10000 rpm for 30 minutes and observed for phase separation if any.

2.5. Antibacterial Activity of Essential Oil Nanoemulsions by Agar Well method:
For this, 100 µl of 24 hrs. old nutrient broth culture of clinical isolates of *S.aureus* (n=70), were inoculated on respective sterilized Hi-sensitivity test Agar plates. Broth culture was spreaded uniformly with sterile cotton swab. Wells were made using sterile borer. To each of the well 100 µl of respective essential oil nanoemulsion were added using micropipette aseptically. All the plates were then kept in refrigerator for 30 minutes so as to facilitate diffusion of nanoemulsion in media along with bacteriostatic action of low temperature in refrigerator. All the plates were then incubated at 37 ºC for 24 hrs.in bacteriological incubator. Next day zone of inhibition of different plates were observed around each nanoemulsions well and zone of inhibition of bacterial growth were measured using zone size measurement scale. (Hi-media), and average value of three replicates were calculated for each isolate and recoded. Agar well studies were also carried out for surfactant Tween 20 and Tween 80. Same procedure is followed for testing antibacterial activity of nanoemulsion formulations

against standard reference culture of *S.aureus* (ATCC 6538). The results obtained were compared statistically using ANOVA test, student's t-test, post hoc tukeys test.

2.6 Measurement of Droplet Size of Nanoemulsions: Essential oil Nanoemulsion formulation from each oil variety showing maximum zone of inhibition was selected for measurement of droplet size and polydispersibility index. It was determined using 90 plus particle size analyzer (ZS, 90 Malvern Instruments, UK). Prior to this each test essential oil nanoemulsion were diluted with deionized water to lower viscosity and multiple light scattering effects.

3. Results and Discussions:

3.1 Physicochemical Characterization: Nanoemulsions with higher surfactant concentration demonstrated higher % Transmittance. Surfactant concentration found to affect the visual appearance of all test oil nanoemulsions except very low effect on Cinnamon oil nanoemulsions. (Table II) This rise in % Transmission may be due to reduction in droplet diameter with increase in surfactant concentration (Chang *et al.*, 2013., Saberi *et al*., 2013). The pH in all 32 nanoemulsions mostly varied in range of 6.0 to 6.5.

3.2 Stability of Nanoemulsions: Increase in surfactant concentration considerably affected the stability of Essential oil nanoemulsions. Stability of test oil nanoemulsions under study was increased by energy input during ultra-sonication. Amongst all eight nanoemulsions of Tea Tree oil 20T2, 20T3, 20T4 was found to be thermodynamically stable when centrifuged at 10000 rpm for 30min. In case of Thyme oil nanoemulsions, 20Th3, 20Th4, &80Th3 exhibited same stability. Whereas Clove oil nanoemulsions 20Cl2 & 80Cl4 s were found to be thermodynamically stable. Cinnamon oil nanoemulsions 20C4 and 80C4 demonstrated stability. (Table II)

Nano emulsion Formulation Code	Visual Appearance	% T	pH	C	H-C*	Nano emulsion Formulation Code	Visual Appearance	% T	pH	C	H-C*
20 T1	Milky White	13	6.0	-	-	20 Cl1	Milky Yellow	14	6.1	-	-
20 T2	Transparent	86	6.0	+	+	20 Cl2	Milky Yellow	15	6.0	+	+
20 T3	Transparent	86	6.0	+	+	20 Cl3	Translucent	18	6.0	-	-
20 T4	Transparent	91	6.0	+	+	20 Cl4	Transparent	85	6.0	-	+
80T1	Milky	16	6.0	+	+	80Cl1	Milky Yellow	12	6.2	-	-
80T2	Milky	14	6.5	-	-	80Cl2	Milky Yellow	14	6.1	-	-
80T3	Translucent	75	7.0	-	+	80Cl3	Milky Yellow	13	6.0	-	-
80 T4	Transparent	90	6.0	+	-	80Cl4	Transparent	85	6.0	+	+
20 Th1	Milky White	12	6.0	+	-	20 C1	Milky	15	6.0	-	-
20 Th2	Milky White	12	6.1	+	-	20 C2	Yellowish White	14	6.0	-	-
20 Th3	Translucent	17	6.0	+	+	20 C3	Yellowish Milky	15	6.0	+	-
20 Th4	Transparent	79	6.3	+	+	20 C4	Off White	14	6.0	+	+
80Th1	Milky White	15	6.0	-	-	80C1	Milky White	15	6.0	-	-
80Th2	Milky White	12	6.0	-	-	80C2	Milky White	17	6.0	-	-
80Th3	Translucent	14	6.0	+	+	80C3	Milky White	14	6.0	-	-
80 Th4	Translucent	14	6.0	+	-	80C4	Yellowish White	12	6.0	+	+

%T= %Transmittance, C- Centrifugation, +=stable, - =unstable

Table II: Physicochemical Characterization and Stability study of Essential Oil Nanoemulsions

3.3 Antibacterial Activity of Nanoemulsion by Agar-Well Diffusion Method:

All formulated Essential Oil Nanoemulsion were screened for its antibacterial activity against clinical isolates of *S.aureus* (n=70) and diameter of zone of inhibition (ZOI) in mm when compared using the data characteristics such as mean, standard deviation, range, etc. were determined and the One-Way Analysis of Variance (ANOVA) procedure was followed for a quantitative dependent variable by a single factor (independent) variable. The tests used were- Post- hoc tukeys test and Students t-test.

3.3.1 Antibacterial activity of Tea tree (*M. alternifolia*) oil Nanoemulsions on *S.aureus*

Table III provides the comparison of diameter measurements of the zone of inhibition (ZOI) across different concentrations of tea tree oil nanoemulsions in *S. aureus* organisms, using a one-way analysis of variance. Across different concentrations of nanoemulsions, 20T1 to 20T4, the mean change in diameter measurement of the ZOI was statistically significantly different with a p-value < 0.0001. Further, a post-hoc analysis using Tukey's test revealed that the mean measurement for 20T2 (16.83 ± 2.13 mm) was statistically significantly higher than the remaining concentrations with p-values less than < 0.0001

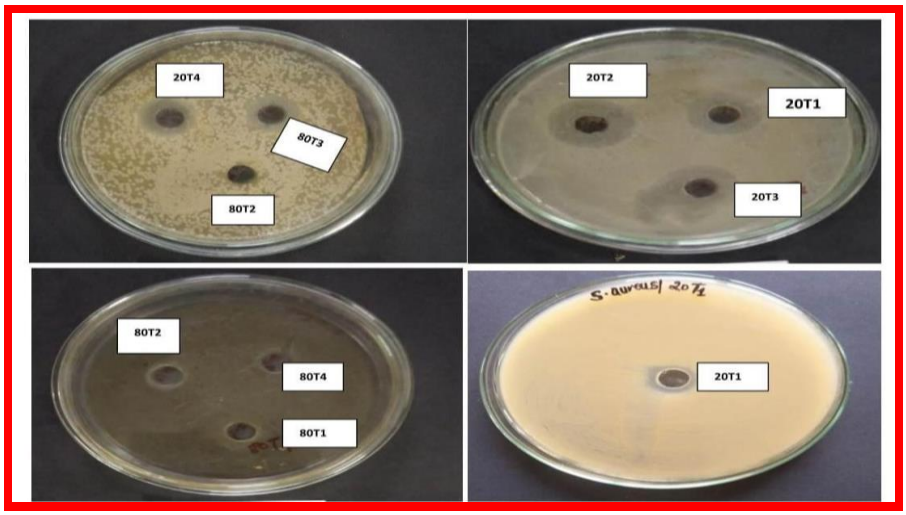


Figure: 1 Antibacterial activity of different nanoemulsions of *M. alternifolia* (Tea tree) oil with surfactants Tween 20 and Tween 80 in varying concentration on *S.aureus* isolate.

Table III: Comparison of diameter measurements of ZOI across different concentrations of tea tree oil nanoemulsions in *S. aureus*

Nanoemulsion code	Diameter of Zone of Inhibition (ZOI)m in mm					P-value*	P-value†
	Reference	Mean	SD	Minimum	Maximum		
20T1	14.00	13.49	1.96	10.00	17.00	< 0.0001 (S)	< 0.0001 (S)
20T2	14.00	16.83	2.13	12.00	22.00		
20T3	12.00	14.28	1.73	10.00	18.00		
20T4	14.00	14.35	1.81	10.00	20.00		
80T1	11.00	13.85	1.39	11.00	17.00	0.1286 (NS)	
80T2	14.00	13.77	1.48	10.00	17.00		
80T3	15.00	14.31	1.59	11.00	17.00		
80T4	13.00	13.94	1.34	12.00	17.00		

3.3.2 Antibacterial activity of Thyme (*Thymus vulgaris*) oil Nanoemulsions on *S.aureus*

Table IV provides the comparison of diameter measurements of the zone of inhibition (ZOI) across different concentrations of thyme oil nanoemulsions in *S. aureus* organisms, using a one-way analysis of variance.

Across different concentrations of nanoemulsions, 20Th1 to 20Th4, the mean change in diameter measurement of the ZOI was statistically significantly different with a p-value 0.0001. Further, a post-hoc analysis using Tukey's test revealed that the mean measurement for 20Th1 (12.32 ± 1.33 mm) was statistically significantly smaller than the concentration 20Th3 (13.31 ± 1.54 mm) and the concentration 20Th4 (13.35 ± 1.67 mm) with p-values 0.0009 and 0.0005 respectively.

For nanoemulsions 80Th1 to 80Th4, the mean diameter measurements were statistically significantly different across nanoemulsions with a p-value < 0.0001. Further, a post-hoc analysis was performed using Tukey's test, which revealed that the mean measurement of nanoemulsion 80Th3 (16.25 ± 1.82 mm) was statistically significantly higher than the remaining nanoemulsions with p-values < 0.0001 (Table 4-m-b). There was also a significant difference between the mean measurements of 80Th1 (13.35 ± 1.17 mm) and 80Th4 (14.54 ± 1.51 mm) with a p-value < 0.0001.

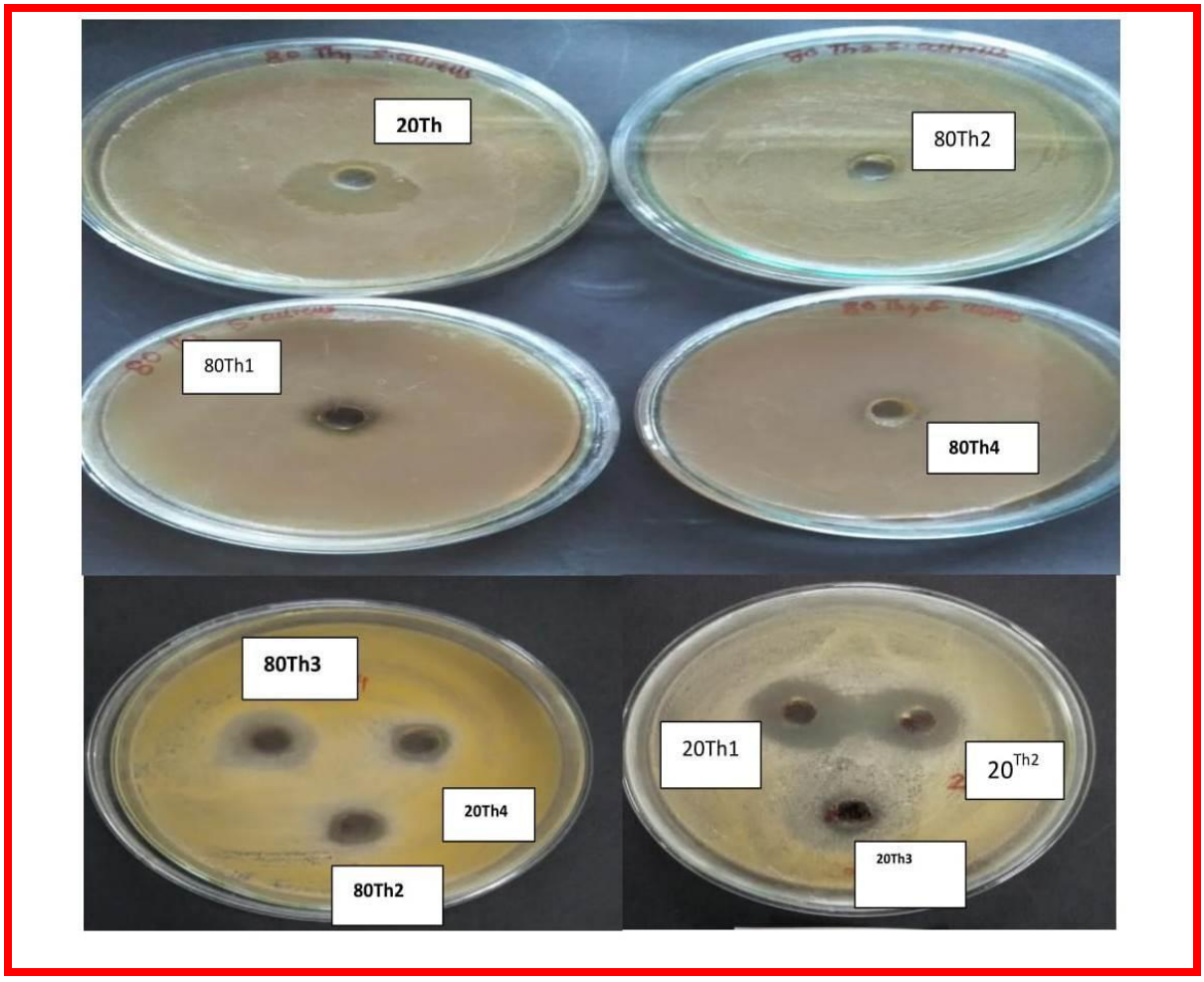


Figure 2: Antibacterial activity of *Thymus vulgaris* (Thyme) oil nanoemulsions with surfactants Tween 20 and Tween 80 in varying concentration on *S.aureus* isolate.

Table IV: Comparison of diameter measurements of ZOI across different concentrations of thyme oil nanoemulsions in *S. aureus*

Nanoemulsions code	Diameter of Zone of Inhibition (ZOI)m in mm					P-value*	P-value†
	Reference	Mean	SD	Minimum	Maximum		
20Th1	13.00	12.32	1.33	11.00	17.00	0.0001 (S)	< 0.0001 (S)
20Th2	12.00	12.83	1.56	10.00	17.00		
20Th3	13.00	13.31	1.54	10.00	16.00		
20Th4	14.00	13.35	1.67	10.00	17.00		
80Th1	13.00	13.35	1.17	11.00	17.00	< 0.0001 (S)	
80Th2	14.00	13.90	1.55	10.00	17.00		
80Th3	15.00	16.25	1.82	13.00	20.00		
80Th4	13.00	14.54	1.51	12.00	19.00		

3.3.3 Antibacterial activity of Clove (*Syzygium aromaticum*) oil Nanoemulsions on *S.aureus*

Across different concentrations of nanoemulsions, 20Cl1 to 20Cl4, the mean change in diameter measurement of the ZOI was statistically significantly different with a p-value < 0.0001. Further, a post-hoc analysis using Tukey's test revealed that the mean measurement for 20Cl2 (16.23 ± 1.24 mm) was statistically significantly higher than the remaining concentrations with p-values less than < 0.0001.

Also, the comparison of nanoemulsions across nanoemulsions 20Cl1 to 80Cl4 were performed. The mean diameter measurements of ZOI were statistically significantly different across nanoemulsions with a p-value < 0.0001. Further, the post-hoc analysis was performed using Tukey's test, which revealed that the mean measurement of nanoemulsion 20Cl1 (14.17 ± 1.61 mm) was statistically

significantly smaller compared to the means of 20Cl2, 20Cl3 and 20Cl4 with p-values less than 0.05. Also, there was a statistically significant difference between 20Cl2 with nanoemulsions 20Cl3, 20Cl4, 80Cl1, 80Cl2, 80Cl3, and 80Cl4 with p-values < 0.0001. The mean measurement of 20Cl3 (14.92 ± 1.71 mm) was statistically significantly higher than nanoemulsions 80Cl2 (14.18 ± 1.46 mm) and 80Cl3 (13.85 ± 1.06 mm) with p-values 0.0218 and < 0.0001 respectively. Further, there was also a significant difference between 20Cl4 with nanoemulsions 80Cl1, 80Cl2, 80Cl3 and 80Cl4 with p-values < 0.05.

Taking into account results obtained from stability of Clove oil nanoemulsions. Nanoemulsion 20Cl2 is selected due to maximum antibacterial activity against clinical isolates of *S.aureus.*

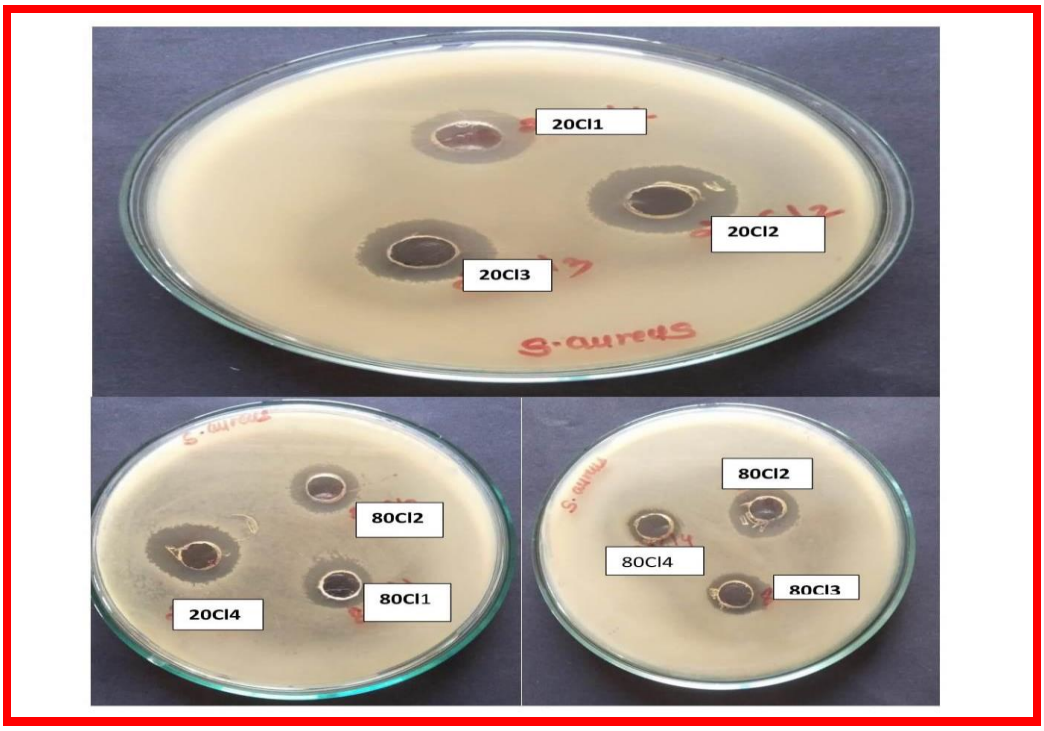


Figure 3: Antibacterial activity of *Syzygium aromaticum* (Clove) oil nanoemulsions *with surfactants Tween 20 and Tween 80 in varying concentration on S.aureus* isolates

Table V: Comparison of diameter measurements of ZOI across different concentrations of Clove oil nanoemulsions in *S. aureus*

Nanoemulsion code	Diameter of Zone of Inhibition (ZOI)m in mm					P-value*	P-value†
	Reference	Mean	SD	Minimum	Maximum		
20Cl1	16.00	14.17	1.61	12.00	17.00	< 0.0001 (S)	< 0.0001 (S)
20Cl2	20.00	16.23	1.24	14.00	20.00		
20Cl3	17.00	14.92	1.71	12.00	17.00		
20Cl4	20.00	14.96	1.16	12.00	20.00		
80Cl1	16.00	14.27	0.97	12.00	16.00	0.0605 (NS)	
80Cl2	19.00	14.18	1.46	12.00	19.00		
80Cl3	15.00	13.85	1.06	12.00	15.00		
80Cl4	13.00	13.86	1.11	12.00	15.00		

3.3.4 Antibacterial activity of Cinnamon (*Cinnamomum zeylanicum*) oil Nanoemulsions on *S.aureus*

Table VI provides the comparison of diameter measurements of the zone of inhibition (ZOI) across different concentrations of cinnamon oil nanoemulsions on *S. aureus*, using a one-way analysis of variance. Across different concentrations of nanoemulsions, 20C1 to 20C4, the mean change in diameter measurement of the ZOI was statistically significantly different with a p-value < 0.0001. Further, a post-hoc analysis using Tukey's test revealed that the mean measurement for 20C4 (33.68

± 1.16 mm) was statistically significantly higher than the remaining concentrations with p-values < 0.0001

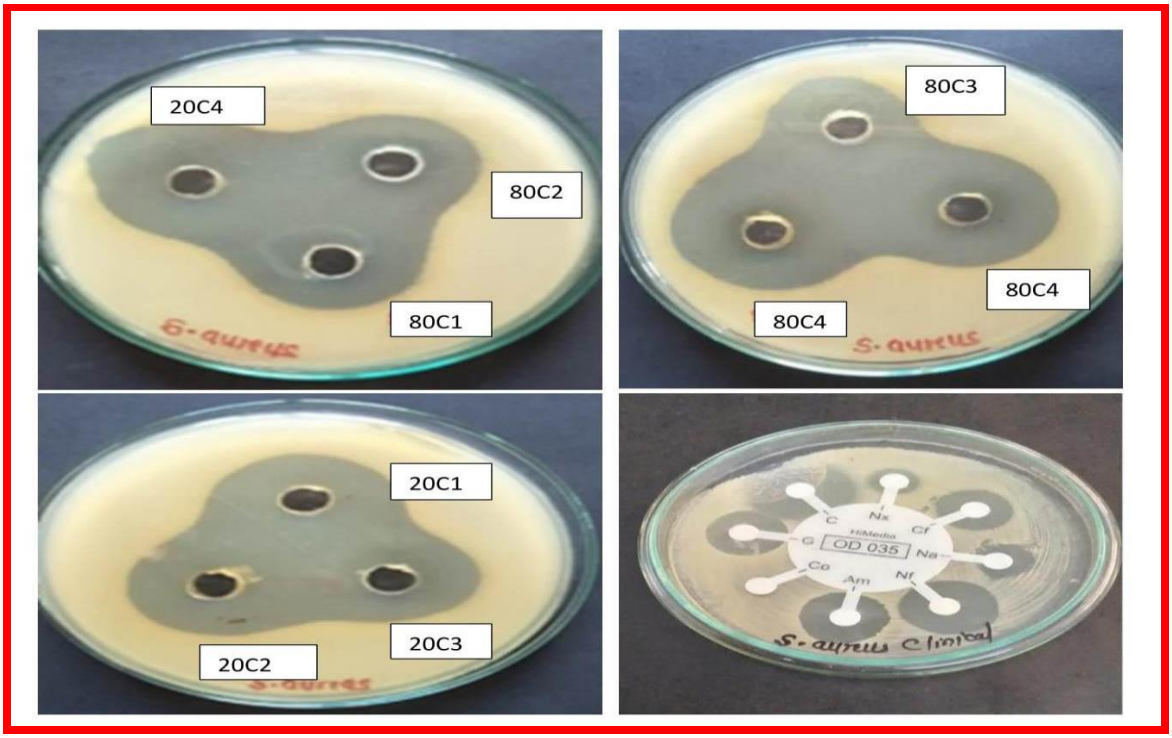


Figure 4: Antibacterial activity of *Cinnamomum zeylanicum* (Cinnamon) oil nanoemulsions with surfactants Tween 20 and Tween 80 in varying concentration on *S.aureus* isolate.

Table VI: Comparison of diameter measurements of ZOI across different concentrations of Cinnamon oil nanoemulsions in *S. aureus*

Nanoemulsion code	Diameter of Zone of Inhibition (ZOI)m in mm					P-value*	P-value†
	Reference	Mean	SD	Minimum	Maximum		
20C1	31.00	29.99	0.78	29.00	31.00	< 0.0001 (S)	< 0.0001 (S)
20C2	30.00	29.75	1.37	20.00	31.00		
20C3	30.00	30.13	0.77	29.00	32.00		
20C4	35.00	33.68	1.16	30.00	35.00		
80C1	34.00	30.28	0.90	29.00	34.00	< 0.0001 (S)	
80C2	34.00	29.69	1.18	28.00	35.00		
80C3	34.00	29.72	0.81	28.00	34.00		
80C4	40.00	35.07	1.10	31.00	40.00		

For nanoemulsions 80C1 to 80C4, the mean diameter measurements were statistically significantly different across nanoemulsions with a p-value < 0.0001. The post-hoc analysis was performed using Tukey's test, which revealed that the mean measurement of nanoemulsion 80C4 (35.07 ± 1.10 mm) was statistically significantly higher than the remaining nanoemulsions with p-values < 0.0001. There was also a significant difference between the mean measurements of 80C1 (30.28 ± 0.90 mm) and 80C2 (29.69 ± 1.18 mm) with a p-value of 0.0031.Figure 4.17 represents antibacterial activity of *Cinnamomum zeylanicum* (Cinnamon) oil nanoemulsions on *S.aureus* isolate. Taking into account results obtained from stability of Cinnamon oil nanoemulsions. Nanoemulsion 20C4 and 80 C4 is selected for further antibacterial activity studies across all nanoemulsions of Cinnamon oil.

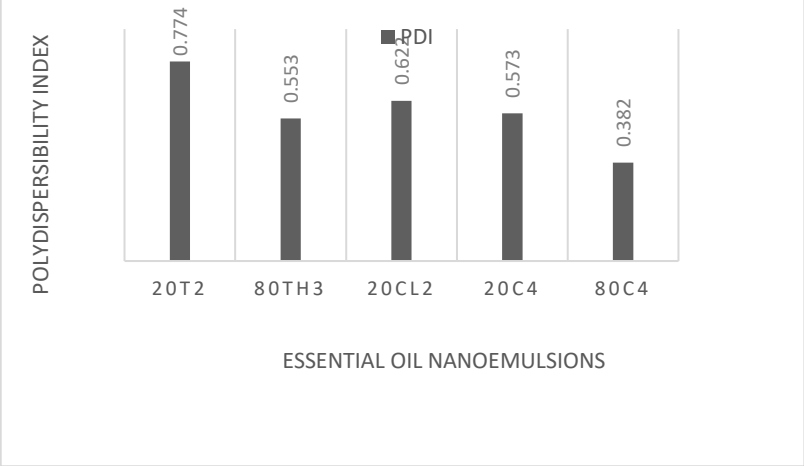
3.4 Measurement of Droplet Size of Nanoemulsions:

The droplet size and pdi of selected nanoemulsions were analyzed using photon correlation microscopy using Malvern zetasizer**.** On comparing polydispersibility index(pdi) and droplet size of selected nanoemulsion formulations showing maximum antibacterial activity from each of test oil type, lowest pdi of 0.382 was reported in Cinnamon oil Nanoemulsion 80C4 with droplet diameter of

133nm ,followed by 20C4 with pdi of 0.573 and droplet diameter of 272.3nm indicating 80C4 and 20C4 as monodispersed as compared to 20T2,80 Th3 and 20Cl2.20T2 nanoemulsion showed highest pdi and droplet diameter in all five nanoemulsions selected for analysis which can be directly correlated with least antibacterial activity of this formulation. Also potential antibacterial activity of Cinnamon oil nanoemulsion formulations can be correlated with its monodispersed nature and lower droplet diameter along with antibacterial content**.** Table VI shows Droplet size measurement of Test Oil Nanoemulsion.

Table VI: Droplet size measurement of Test Oil Nanoemulsion

Nanoemulsions code	Pdi Index	Droplet diameter (nm) z-average
20T2	0.774	460.8
80Th3	0.553	285.9
20Cl2	0.622	303.3
20C4	0.573	272.3
80C4	0.382	133.6



Graph I Droplet size measurements of essential Oil Nanoemulsions

4. Discussion:

On comparison of antibacterial activity of all 32 nanoemulsions formed using Tea tree, Thyme, Clove leaf & Cinnamon oil. Maximum antibacterial activity (in terms of mean of zone of inhibition (mm)), against clinical isolates of *S.aureus* were demonstrated by stable nanoemulsions 20T2, 80Th3, 20 Cl2, 20C4, and 80C4 respectively in each of oil type. Comparison of mean of diameter of zone of inhibition obtained in these nanoemulsion formulations demonstrated maximum antibacterial activity in Cinnamon oil Nanoemulsion formulation 80C4 with mean value of zone of inhibition of 35.07 mm, followed by 20C4 of 33.68 mm, against clinical cultures of *S.aureus*. Nanoemulsion formulations 20T2, 80Th3, and 20Cl2 was also found to exhibit antibacterial effect in agar well diffusion experiment but not to the extent of that of Cinnamon oil Nanoemulsion formulations. The potent antibacterial activity of Cinnamon oil nanoemulsion may be due to the potent antibacterial constituents of cinnamon oil, as well as reduced droplet diameter of cinnamon oil nanoemulsions as compared to that of nanoemulsions of other oils under study. The results obtained corroborates with findings of Chandrshekhran *et al*. (2013). Who reported that cinnamon oil nanoemulsions demonstrates potent antibacterial activity .The antibacterial activity results obtained also corroborates with the findings of Donsi *et al*.,2012, who reported that ,Cinnamaldehyde nanoemulsion greatly inhibits bacterial pathogens.

5. Conclusion:

Cinnamon oil based stable nanoemulsions- 20C4 & 80C4 containing Tween 20 and Tween 80 as surfactant with droplet diameter in 272.3 nm and 133.6 nm demonstrated maximum bactericidal activity against clinical isolates of *S.aureus.* Thus cinnamon oil can be exploited to prepare natural potent antibacterial nanoemulsions against *S.aureus.*

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