RESEARCH ARTICLE

Preparation and characterization of O/W nanoemulsion with eucalyptus essential oil and study of *in vitro* antibacterial activity

Arun Dev Sharma*, Mohit Farmaha, Inderjeet Kaur

PG Department of Biotechnology, Lyallpur Khalsa College Jalandhar, Punjab, India

ARTICLE INFO

Article History:
Received 25 July 2020
Accepted 02 Sep 2020
Published 01 Nov 2020

Keywords:
Nanoemulsion
Eucalyptus Oil
Tween 20
In virto antimicrobial activity

ABSTRACT

Objective(s): Eucalyptus essential oil is widely used pharmaceutical, medicine and ornamental industries having antimicrobial as well as antioxidant activities. Eucalyptus oil is highly unsteady under ordinary conditions, so simply lose its bactericidal action. To protect this bioactive herbal product, nano-based emulsion technology is an outstanding method to micro-encapsulate and hydrophilize it. Due to special properties, oil-in-water (O/W) essential oil nanoemulsionsare expanding day by day as a delivery system in food, cosmetic and agrochemical industries. This study prepare eucalyptus oil based nanoemulsions, its characterization and *in vitro* evaluation of its antimicrobial activity.

Methods: O/W nanoemulsionswere prepared by using eucalyptus essential oil in the presence of surfactants like tween 20, tween 80 and sodium dodecyl sulphate (SDS). Physiochemical parameters like pH, conductivity, optical density and antibacterial activity were studied. Stability of nanoemulsions was studied for 120 days.

Results: Nanoemulsions prepared with tween 20/80 and SDS were smaller in size than those prepared with tween 20/80. Formulated nanoemulsions were stable even after 120 days as no significant change in pH, conductivity, size of droplet was observed. Substantial *in vitro* antibacterial activity against *Bacillus subtilis* was observed with nanoemulsions prepared with tween 20 only.

Conclusion: The findings suggested that eucalyptus oil based nanoemulsions can be used as key drug carrier in pharmaceutical, food and cosmetic industries.

How to cite this article
Sharma AD, Farmaha M, Kaur I. Preparation and characterization of O/W nanoemulsion with eucalyptus essential oil and study of in vitro antibacterial activity. Nanomed Res J, 2020; 5(4): 347-354. DOI: 10.22034/nmrj.2020.04.006

INTRODUCTION

Plant essential oils are secondary metabolites from aromatic plants. Essential oil is a complex mixture of various bioactive chemical molecules such as terpenes, esters, alcohols and other aromatic compounds [1]. Due to these diverse complex bio-structures which act synergistically, essential oil posse various biological activities like antimicrobial, antiviral, fungicidal, insecticidal and herbicidal. Due to herbal nature and safer alternative to chemical drugs, interest in these kinds of plant based secondary metabolites is increasing day by day. Due to having antioxidant and antimicrobial activities, essential oils are of great potential in medicine industries. Consequently, with time an immense attention has been given to essential oils as curative medicines [2]. *Eucalyptus globules,* member of Myrtaceae family, is native to Australia and Tasmanian member extensively spread genera known for its biological and pharmaceutical properties. The leaves of this plant are used to extract eucalyptus oil (EO, *Oleum Eucalypti*) worldwide. Essential oil from this aromatic plant has long history to be used as traditional medicine in ancient times. Due to enriched presence of 1,8-cineole (eucalyptol), essential oil from this

* Corresponding Author Email: *arundevsharma47@gmail.com*

plant is widely used as raw material in cosmetic, pharmaceutical and food industries[2]. Although EO has been empirically used as antimicrobial agent but its mechanism of action is still in infancy.

In recent times, encapsulation of EOs as nanoemulsions has been proved as a new method to enhance its efficiency, stability and utilization [3]. In this direction, nanotechnology has paved a significant progress in various scientific fields and innovation. In recent times, nanoemulsions have been extensively studied and offer substantial potential as functional ingredient in cosmetic, pharmaceutical, food and agriculture products[4]. Nanoemulsions are divided into following categories like oil-in-water (O/W), water-in-oil (W/O), transparent/translucent and colloidal dispersions[5]. Nanoemulsions are effective to deliver hydrophobic bioactive compounds having aroma and flavors with increased surface area. Therefore, the lipophilic bioactive components can be included in oil phase of nanoemulsion and aqueous medium in the presence of emulsifier like Tween 20 and Tween 80 [6]. Tween-20 and 80 are nonionic, biocompatible, and non-toxic surfactants and widely implicated in medicine, cosmetic, food industries products as used as emulsion surfactants. [7]. Eucalyptus oil is one among hundreds of different types of essential oils and due to presence of 1-8 cineole (eucalyptol), EO posses various activities like antimicrobial, insecticide and expectorant. However, 1-8 cineole (eucalyptol) is highly volatile under normal conditions thus unstable and susceptible to oxidative degradation by air and eventually loss of oil integrity and antimicrobial action [8]. Therefore, nanoemulsion technology offers nice alternative to encapsulate, hydrophilize and protect this bioactive compound. It can be used as oil soluble part of O/W nanoemulsions. By this method concentration of phytoactive compounds like eucalyptol in microorganism rich areas can be enhanced. Hence, the present study aimed at to form nanoemulsions of eucalyptus oil and investigation of antimicrobial activity. This work will endow with new information about nanoemulsions based on eucalyptus essential oil in different areas such as food, cosmetic, and pharmaceutical drug delivery systems.

MATERIAL AND METHODS

Extraction of eucalyptus essential oil

The leave samples of *Eucalyptus globules* were collected growing under natural conditions, from nearby areas of Lyallpur Khalsa College, Jalandhar, located at 71^0 - 31^0 east latitude and 30^0 -33^0 north longitude. The city experiences humid subtropical climate with hot and cold months. The climate is dry on the whole. The experiment was conducted on February 2020.

Steam-distillation method was used for extracting of essential oil. Extraction was performed using Clevenger-type apparatus. About 15 g of crushed green leaves were distilled at a working temperature of 100°C for 2 hr with addition of 100 ml of double-distilled water. Extraction of oil continued until no more oil was retried. The oil was collected from Clevenger apparatus and stored at laboratory condition (25°C). The oil obtained was used without further purification or filtration.

Preparation of Nanoemulsion

Nanoemulsions of *Eucalyptus globules* essential oil were prepared by method given by Moradi and Barati [9] (Table 1). Briefly, for the preparation of EO based nanoemulsions, Tween 80 (2v/v%) and Tween 20 (2v/v%) were dissolved in double distilled water at room temperature. The mixture was vortexed for 15 minutes to get homogenous solution. Then eucalyptus essential oil was added slowly to the above said mixture and shaken with magnetic stirrer for 15 minutes. Then mixture was allowed to stir with magnetic stirrer for further 10 minutes.

In other experiment, SDS (0.25 w/w%) was added to mixture containing Tween 80 (2v/v%) and Tween 20 (2v/v%) and distilled water. The mixture was allowed to stir with magnetic type

Table 1: Composition of nanoemulsion components

Sample name	Essential oil Type	Essential oil (%v/v)	Tween 20 (%v/v)	Tween 80 (%v/v)	SDS (%w/v)
EG1	*Eucalyptus globulus*	2	2	-	-
EG2	*Eucalyptus globulus*	2	-	2	-
EG3	*Eucalyptus globulus*	2	2	-	0.25
EG4	*Eucalyptus globulus*	2	-	2	0.25

stirrer for 10 minutes. Similarly, essential oil was added slowly to the mixture for 15 minutes with continue stirring. Then mixture was allowed to stir with magnetic stirrer for further 10 minutes. Nanoemulsion composition prepared in this study is described in Table 1.

Characterization

Nanoemulsion properties

Physiochemical characterization studies were administered to characterize and study the physical stability of the formulated nanoemulsions. The pH values of the nanoemulsions were measured by immersing the pH electrode into the undiluted emulsion using pH meter (Labtronics). Conductivity of the nanoemulsions formulations was measured by using conductometer (Labtronics). All the values were determined at room temperature.

Turbidity

For the turbidity, the absorbance of the nanoemulsions was measured at 600 nm using UV-VIS spectrophotometer (Labtronics). All measurements were recorded three times for each sample and the average values were reported.

Size

Size of the formulated nanoemulsions was observed with the help of the microscope (Debro DM RL) having 5 MP 1 / 2.5 APTINA CMOS sensor. The size of the nanoemulsion was noted with the help of Micro View software with inbuilt calibration tools.

In vitro Antibacterial activity of O/W nanoemulsions

In vitro antibacterial activity of the *Eucalyptus globules* essential oil and nanoemulsions were carried out by agar disc diffusion method against test organism (gram-positive bacteria *Bacillus subtilis* MTCC 121). A swab of bacteria suspension was spread on to the petri plates having Luria Broth. Sterile paper discs (6 mm in diameter) impregnated with *Eucalyptus globules* essential oil nanoemulsions were placed on culture plates and incubated at 37^0 C for 24 hours. Nanoemulsions without essential oil were taken as negative control while standard vancomycin (5 mg) discs were served as positive control. Zone of inhibition was calculated which indicates antibacterial activity.

Statistical analysis

MS Excel software was used to determine P values by student t test. Values of P< 0.05 were significant.

RESULTS AND DISCUSSION

Physiochemical properties

EO nanoemulsions were characterized by measuring their physiochemical properties like pH and conductivity at room temperature(Table 2).Conductivity is the ability of any molecule to transfer electricity between two points.To check the stability and then nature of formulation, electrical conductivity of the nanoemulsions was determined [10]. It provides information about nanoemulsions continuous phase and phase inversion phenomenon [11]. As shown in Table 2, conductivity of EO nanoemulsions prepared with Tween 20 and Tween 80 showed almost equal conductivity. However, when surfactants were blended with SDS, increased conductivity of nanoemulsions was observed. This is quite feasible as conductivity is directly linked to the amountofions. Conductivity increased as the amount of ions increased. Turbidity of the formulated nanoemulsions was noted at 600 nm with UV-VIS spectrophotometer. It determines qualitative and quantitative analyses of samples [12]. UV-VIS evaluates amount of light absorbed by the bioactive sample by measuring light intensity. The results of UV-VIS analysis is presented in Table 3. As per Abs600 results, addition of SDS increased Abs at 600. Tomaszewsk et al [13] cited that optical properties of nanoemulsion are very sensitive to size, shape and concentration changes. Increase in Abs600 indicated that amount of nano-particles increased which led to increase in the absorbance. Size and the pictures of the

Table 2: Physiochemical properties of *Eucalyptus globulus* essential oil nanoemulsions

Sample name	EG1	EG2	EG3	EG4
Abs 600	1.735 ± 0.015	2.174 ± 0.005	2.459 ± 0.045	2.312 ± 0.040
Abs 600 (After 4 month storage)	1.658 ± 0.049	2.072 ± 0.058	2.366 ± 0.015	2.266 ± 0.015

Table 3: Turbidity of formulated nanoemulsions at 600 nm

Sample name	EG1	EG2	EG3	EG4
Abs 600	1.735 ± 0.015	2.174 ± 0.005	2.459 ± 0.045	2.312 ± 0.040
Abs 600 (After 4 month storage)	1.658 ± 0.049	2.072 ± 0.058	2.366 ± 0.015	2.266 ± 0.015

Table 4: Size of formulated nanoemulsions

Sample name	Size nm (After preparation)	Size nm (After 4 months of storage)
EG1	474 ± 88.08	496 ± 89.76
EG2	628 ± 88.79	705 ± 92.64
EG3	260 ± 48.30	293.5 ± 53.75
EG4	368 ± 87.78	408 ± 77.28

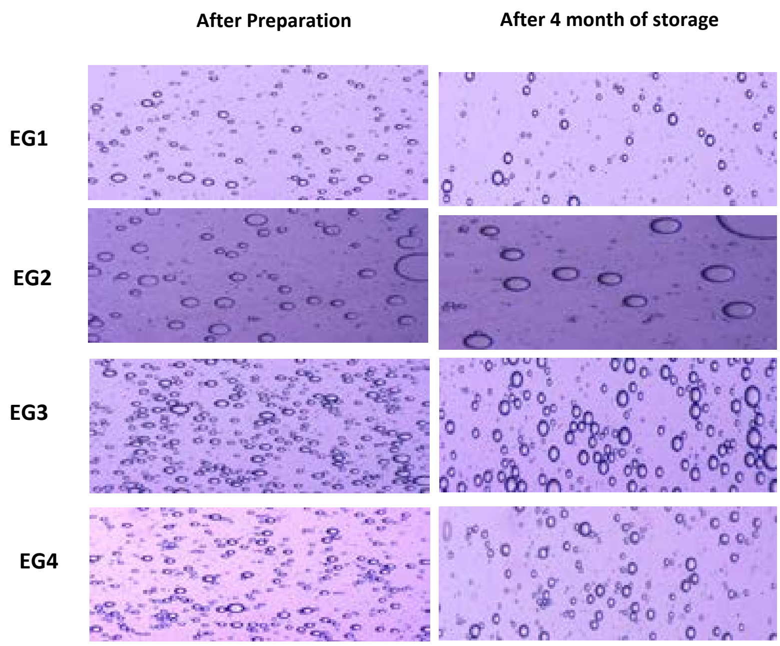

Fig. 1: Microscopic view of eucalyptus essential oil based nanoemulsions. EG1: Tween 20 + Oil (10X), EG2= Tween 80 + Oil (10X), EG3= Tween 20 + Oil + SDS (10X), EG4= Tween 80 + Oil + SDS (10X)

formulated nanoemulsions after preparation and after 4 month of storage are illustrated in the Fig. 1 and Table 4. It can be inferred that most of nanoemulsion droplets were almost spherical in shape. Mean droplet size of nanoemulsions prepared with tween 20 was smaller than tween 80. It can be due to difference in molecular weight of surfactants (MW tween 20<MW tween 80). The size of the formulated nanoemulsions indicated that the nanoemulsion made with SDS (EG3 and EG4) had less size as compared to nanoemulsion made without SDS (EG1 and EG2). The results of our study are supported by Moradi *etal,*[9].I t was also observed that the number of nanoemulsions was more in the case of nanoemulsions formulated with SDS (EG3andEG4)than nanoemulsions formulated without SDS (EG3 and EG4) (Fig.2). Our observations are supported by Silva *et al*, [14]. It was argued that small molecule emulsifier like SDS can form small particles under same process conditions. It can be assigned to differences in adsorption rate and interfacial properties like charge, thickness, permeability and environmental responsiveness [14]. Qian and McClements [6]

also cited that emulsifiers with higher adsorption rate and smaller interfacial forces lead to smaller particle sizes.

Storage Stability

One of the major factors in preparation of nanoemulsions is their stability over the time. For using as anti-microbial or anti-bacterial agents in delivery system, long term stability is prerequisite. No substantial change in conductivity was observed after 4 months of storage at room temperature. If the conductivity stays stable after storage at room temperature, itindicates nanoemulsion stability without phase inversion [15]. The pH value of the nanoemulsion is very important to determine its stability. The change in the pH is indication of the chemical reaction occurrence that can affect the quality of final product [16]. As shown in the table 2, the pH values are hardly changed over the time, indicates the stability of the nano emulsions. Ostwald repining which the major aspect of preparation of essential oil nanoemulsions against was also studied by measuring droplet size after storage. Ostwald repining is the conversion of small droplets to larger ones due to diffusion of oil molecules through intervening aqueous phase [17]. No substantial increase in size of nanoemulsions was recorded. The formulated nanoemulsions were physically stable over the 4- months of storage at room temperature which makes them interesting candidate for the practical applications.

Antibacterial properties of Eucalyptus globulus essential oil and its Nanoemulsions

Eucalyptol (1,8 cineole), owing to lipophilic in nature, has the capability to penetrate plasma cell membrane of bacteria. Eventually it leads to breakdown of cell membrane integrity and seepage of cellular components followed by cell death[18]. The antiseptic activity of pure essential oil and in nanoemulsion form was measured against gram-positive bacteria *Bacillus subtilis*. The anti-bacterial properties of *Eucalyptus globulus* essential oil improved significantly when it was transformed into nanoemulsion. It may be accredited to easier admittance of essential oil to the bacterial cells. It is plausible that nanoemulsion appeared to amplify the antibacterial activity of eucalyptus essential oil possible by increasing their ability to disrupt cell membrane integrity. Moghimi et al. [3] by

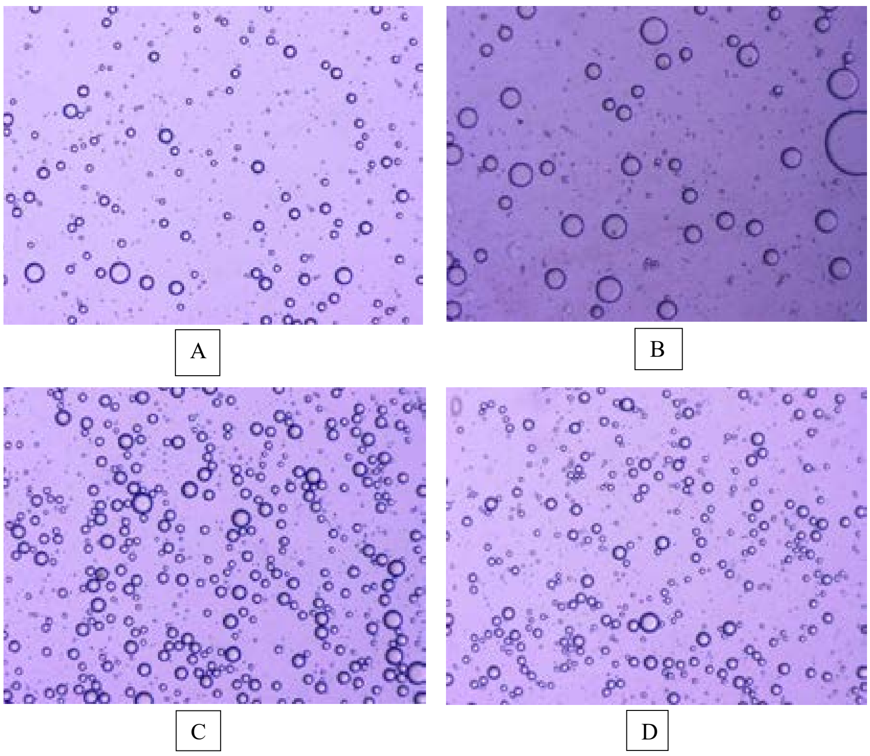

Fig. 2: Pictures of formulated nanoemulsions A= Tween 20 + Oil (10X), B= Tween 80 + Oil (10X), C= Tween 20 + Oil + SDS (10X), D= Tween 80 + Oil + SDS (10X).

electron microscopy also cited that essential oil nanoemulsion killed more bacteria cells than pure oil as evident by measuring increase in cytoplasmic contents like protein, potassium and nucleic acids. It was noted that nanoemulsion made with Tween 20 and *Eucalyptus globulus* essential oil (EG1) strongly inhibited the growth of bacteria (zone of inhibition 1.62 cm) than other formulated nanoemulsions (EG2, EG3, EG4) (Fig. 3). The antibacterial activity was in according to earlier reports from researchers [3]. Nanoemulsions can keep essential oils from evaporation and decomposition during heat, sunlight and pressure [19]. Nanoemulsions made with Tween 80 and *Eucalyptus globulus* essential oil (EG2) did not show any inhibition (Fig.4B). As shown in Fig. 4D, nanoemulsions made with Tween 20, *Eucalyptus globulus* essential oil and SDS (EG3) showed the antibacterial properties. It was also noted that blank plate also showed antibacterial inhibition (Fig 4C). This is may be due to the fact that SDS has antibacterial property [17]. As shown in Fig. 4 (F), it was noted that nanoemulsions formulated with Tween 80,*Eucalyptus globulus* essential oil and SDS (EG4) showed very minor inhibition of bacteria. Literature revealed that the antibacterial

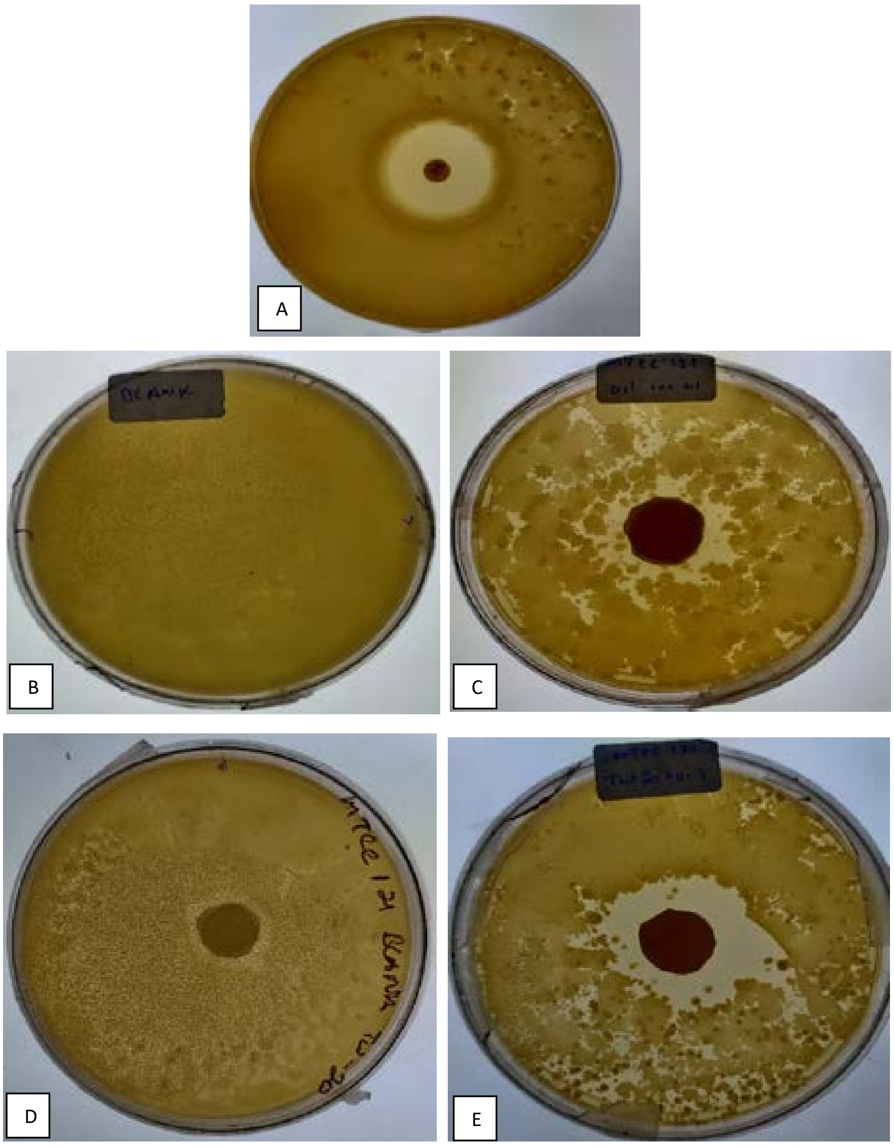

Fig. 3: Antibacterial property of Eucalyptus globulus essential oil and formulated nanoemulsion. A = Vancomycin (Positive control), B= Blank (Without Oil), C= *Eucalyptus globulus* essential oil, D= Blank (Tween 20), E= Tween 20 + Oil

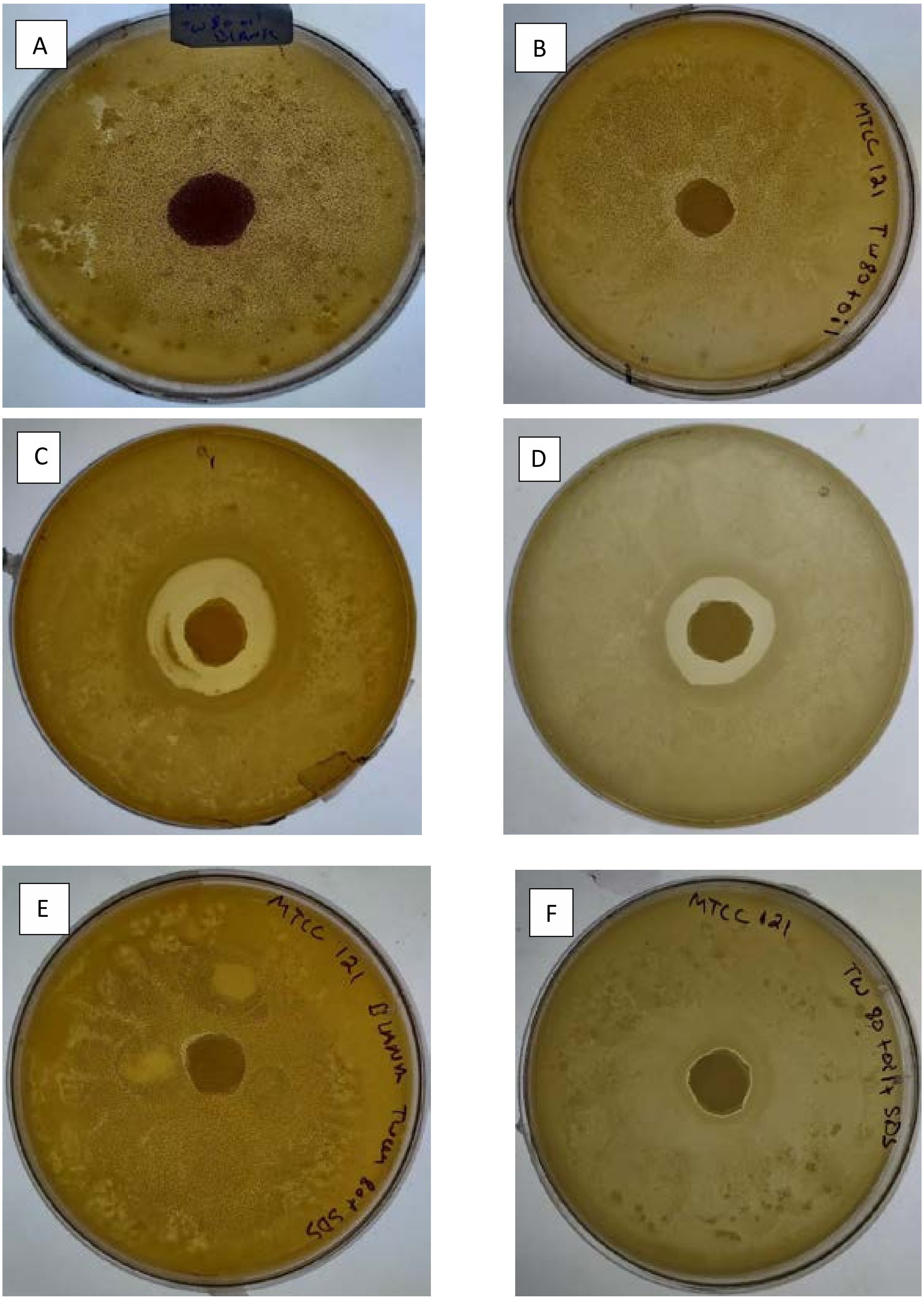

Fig. 4: Antibacterial property of Nanoemulsions of *Eucalyptus globulus* essential oil. A = Tween 80 (Blank), B = EG2 (Tween 80 + Oil), C = Tween 20 + SDS (Blank), D = EG3 (Tween 20 + Oil + SDS) , E= Tween 80 + SDS (Blank), F = EG4 (Tween 80 + Oil + SDS).

properties of nanoemulsions highly depend upon the formulation of the nanoemulsions[9].

CONCLUSION

EO based nanoemulsions have obtained greater interest as pharmaceutical, drug delivery, food products and cosmetic formulations. Nanoemulsions of *Eucalyptus globulus* essential oil and different surfactants were prepared and investigated in this study. Various physiochemical properties of formulated nanoemulsions such as pH, size and conductivity were measured after preparation and after 4 months of storage to check nanoemulsions stability. The physical stability was good and suitable for 120 days at room temperature with not much phase separation or particle growth. Eucalyptus essential oil showed substantial antibacterial activity in the presence of tween 20 against *B. subtilis*. The prepared eucalyptus oil based nanoemulsion could be a cost effective method with apt potential in an array of applications in variety of fields like food, cosmetic and pharmaceutical in future.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

Funding

This work has been funded by DST, seed Govt of India.

REFERENCES

1. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. Asian Pacific Journal of Tropical Biomedicine. 2012;2(9):739-42.
2. Goodger JQD, Senaratne SL, Nicolle D, Woodrow IE. Correction: Foliar Essential Oil Glands of Eucalyptus Subgenus Eucalyptus (Myrtaceae) Are a Rich Source of Flavonoids and Related Non-Volatile Constituents. PLOS ONE. 2016;11(5):e0155568.
3. Moghimi R, Ghaderi L, Rafati H, Aliahmadi A, McClements DJ. Superior antibacterial activity of nanoemulsion of Thymus daenensis essential oil against E. coli. Food Chemistry. 2016;194:410-5.
4. Guglielmini G. Nanostructured novel carrier for topical application. Clinics in Dermatology. 2008;26(4):341-6.
5. Al-Edresi S, Baie S. Formulation and stability of whitening VCO-in-water nano-cream. International Journal of Pharmaceutics. 2009;373(1-2):174-8.
6. Qian C, Decker EA, Xiao H, McClements DJ. Nanoemulsion delivery systems: Influence of carrier oil on β-carotene bioaccessibility. Food Chemistry. 2012;135(3):1440-7.
7. Kerwin BA. Polysorbates 20 and 80 Used in the Formulation of Protein Biotherapeutics: Structure and Degradation Pathways. Journal of Pharmaceutical Sciences. 2008;97(8):2924-35.
8. Turek C, Stintzing FC. Stability of Essential Oils: A Review. Comprehensive Reviews in Food Science and Food Safety. 2013;12(1):40-53.
9.Moradi S, Barati A. Essential Oils Nanoemulsions: Preparation, Characterization and Study of Antibacterial Activity against Escherichia Coli. Int. J. Nanosci. Nanotechnol, 2019;15: 199-210.
10.Talegaonkar S, Tariq M, Alabood R M.Design and Developement of O/W Nanoemulsion for the Transdermal Delivery of Ondansetron. Bull. Pharm. Res.,2011; 1: 18-30.
11. Guglielmini G. Nanostructured novel carrier for topical application. Clinics in Dermatology. 2008;26(4):341-6.
12.Abdelhalim M K, Mady M M, Ghannam MM. Physical Properties of Different Gold Nanoparticles: Ultraviolet-Visible and Fluorescence Measurements. Nanomed. Nanotechnol.,2012;3: 1-5.
13. Tomaszewska E, Soliwoda K, Kadziola K, Tkacz-Szczesna B, Celichowski G, Cichomski M, et al. Detection Limits of DLS and UV-Vis Spectroscopy in Characterization of Polydisperse Nanoparticles Colloids. Journal of Nanomaterials. 2013;2013:1-10.
14. Silva HD, Cerqueira MA, Vicente AA. Influence of surfactant and processing conditions in the stability of oil-in-water nanoemulsions. Journal of Food Engineering. 2015;167:89-98.
15.Talegaonkar S, Tariq M, Alabood R M. Design and Developement of O/W Nanoemulsion for the Transdermal Delivery of Ondansetron.Bull. Pharm. Res., 2011;1: 18-30.
16. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, et al. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. Journal of Nanobiotechnology. 2011;9(1):44.
17. Ziani K, Chang Y, McLandsborough L, McClements DJ. Influence of Surfactant Charge on Antimicrobial Efficacy of Surfactant-Stabilized Thyme Oil Nanoemulsions. Journal of Agricultural and Food Chemistry. 2011;59(11):6247-55.
18. Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. Evidence-Based Complementary and Alternative Medicine. 2016;2016:1-21.
19. Marei G, Rabea E, Badawy M. Ultrasonic Emulsification and Characterizations of Bio-based Nanoemulsion Formulations Containing Citral with Their Antimicrobial Activity. Egyptian Academic Journal of Biological Sciences, F Toxicology & Pest Control. 2017;9(3):169-82.