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Original article

Garlic and ginger essential oil-based neomycin nano-emulsions as effective and accelerated treatment for skin wounds' healing and inflammation: *In-vivo* and *in-vitro* studies

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ABSTRACT

Skin, largest organ of human, is directly exposed to environment and hence is prone to high rates of injuries and microbial infections. Over the passage of time these microbes have developed resistance to antibiotics making them ineffective especially in lower doses and hence, higher dosages or new drugs are required. The current study deals with designing of nano-emulsion (NE) formulations composed of garlic and ginger oils (0.1 %) with neomycin sulphate used in different ratios (0.001, 0.01 and 0.1 %) and combinations. The resulting NEs were characterized for droplet size (145–304 nm), zetapotential (-3.0–0.9 mV), refractive index (1.331–1.344), viscosity (1.10–1.23cP), transmittance (96–99 %), FT-IR and HPLC and found stable over a period of three months. All NEs were also found effective against both gram positive and negative bacterial strains i.e., *B. spizizenii*, *S. aureus*, *E. coli* and *S. enterica* as compared to pure neomycin sulphate (NS) used as control with highest activity recorded for NE-2 and NE-4 against all strains showing zone of inhibition in range of 22–30 mm and 21–19 mm, respectively. NEs were also tested using rabbit skin excision wound model which potentiates that all the NEs resulted in early recovery with 86–100 % wound healing achieved in 9 days as compared to NS ointment (71 %). The studies confirmed that essential oils when used in combination with traditional drug can lead to much higher efficacies as compared to pure drugs.

1. Introduction

Skin, an important and largest organ of human body, is considered most vulnerable to impairment, injury, scratches, burns and bacterial infections in aspect that it is the only organ of body which is directly exposed to environment. Wound healing is one of the most complexed physiological processes involving multiple cells, processes and recovery time periods depending upon age group, infection level and comorbidities. Crack up of this complicated path way is culpable for failure of wound healing resulting in pyogenic wound infections (Ahmad et al. 2019b; Negut et al. 2018; Rijal et al. 2017) owing to development of microorganisms (Negut et al. 2018; Rijal et al. 2017) that if not tackled well in time can cause morbidity and in worst cases even to mortality.

Abbreviations: NE, Nano-emulsion; NS, Neomycin sulphate; ATR, Attenuated Total Reflection; DS, Droplet Size; ZP, Zeta Potential; PDI, Poly dispersity index; RSD, Relative Standard Deviation; WH, Wound Healing; RI, Referective index; T, Transmittance; FT, Freeze–thaw; ZOI, Zone of inhibition; HC, Heat-cool; C, Centrifuge.

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Over the period of time, microorganisms have developed multidrug-resistance and this have impacted the hype in use of antibiotics doses as well as health care cost. In-spite of all that the significant increase in morbidity and mortality cannot be negated. One such microbe that is associated with impaired wound healing is *Staphylococcus aureus* (Dai et al. 2010; Elgayyar et al. 2001; Rijal et al. 2017) which if present impacts wound closure owing to presence of extracellular protein which promotes biofilm formation (Athanasopoulos et al. 2006; Schierle et al. 2009; Yazdani et al. 2006). Development of antibiotic resistant strains have put the efficacy of current therapeutic drugs in question which are proven to be not much helpful and there is a dire need to search for more effective approaches that in addition to being efficient are also cost-effective and result in early recovery of wounds.

Medicinal plants as well as their essential oils are deemed of high importance for treatment of wounds and other diseases. In addition to that they are considered good and safe alternative to current therapeutic drugs (Costa et al. 2019; Woollard et al. 2007). In this regard several oils (thyme (Altiok et al. 2010), oregano (Süntar et al. 2011), garlic (Sidik et al. 2006), neem oil (Rahmani et al. 2018), ginger (Khan et al. 2020) etc.) (Table S1) have been exploited and owing to their high antimicrobial activities these essential oils have drawn scientific as well as commercial interest as potential compounds for development of new drugs and ointments. Moreover, the ease of availability of raw materials make them highly probable materials for designing of low-cost medicines (Costa et al. 2019).

Considering these factors, the current study is aimed to explore the antimicrobial, antioxidant, wound healing and anti-inflammatory potential of the two commonly available house hold items i.e., garlic (*Allium sativum*) and ginger (*Zingiber officinale*). Garlic and ginger are known to be natural antibiotic and versatile healer with antimicrobial activity against multifarious bacteria and fungi (Adetumbi and Lau 1983; Pakrashi and Pakrashi 2003). The essential oils of these are equally beneficial medicinally and have been used for treatment of many diseases (Bordia et al. 1977; Jeena et al. 2015; Lai et al. 2014; Liu et al. 2013) owing to their anti-ulcer, anti-inflammatory, anti-diabetic property, immunity booster, blood circulation, antimicrobial activity, antioxidant, anti-cancer and anti-cramp effects (Imo and Za'aku 2019). Garlic oil is loaded with 84.3–98.9 % bioactive dialkyl polysulphides sulphur containing organic components (Dziri et al. 2014). On other hand ginger essential oil is equipped with zingiberene, curcumene, β-bisabolene, and β-sesquiphellandrene (Wohlmuth et al. 2006).

The efficacy of these oils can be considerably enhanced by exploiting their potential at smaller particle sizes and as carrier for drugs (Valizadeh et al. 2018). In drug delivery system, nanotechnology have already shown significant potential for the enhancement of pharmacological effects and pharmacokinetic profile of bioactive materials of plant and drug molecules (Ahmad et al. 2019b). Nano-emulsion of essential oils have been used effectively to cater many problems especially the drug resistant microbes and in much smaller concentrations as compared to traditional therapeutic doses (Katata-Seru et al. 2017; Moghimi et al. 2018) thereby reducing the cost as well as the negative impact of high dosages. Furthermore, these nano-emulsions have great potential to be exploited as vehicle for drug delivery as they offer high stability, improved bioavailability, high permeation through skin, and better uptake. This opens up new era of discovery in the domains of skin care products as well as pharmaceutical formulations through which drugs with anti-inflammatory, wound-healing and sun-protection activities can be easily delivered through tropical delivery (Franklyne et al. 2021; Souto et al. 2022; Wilson et al. 2022).

Hence, the current study is focused on use of easily available house-hold items i.e., garlic and ginger by exploiting their potential at nano-scale and employment of minimal dosages that are compared with traditional antibiotic drug (Neomycin sulphate) used to treat the skin wounds and infections. In order to fully understand the impact of these nano-emulsions in tissue repair and early recovery of wounds studies were carried out in experimental animals to adequately configure human conditions and corroborate the treatment in actual *in-vivo* studies.

2. Materials and methods

2.1. Materials

Ethylene glycol (CAS no: 107–21-1, 99.8 %) and Tween 80 (Polyoxyethylenesorbitan monooleate) surfactant (CAS:9005–65-6) were procured from Sigma-Aldrich. Neomycin sulfate AI (X3-180602–1, 97.54 %, QC No: R1902092) was supplied by local pharmaceutical company while Neomycin sulfate-based ointment was purchased from local drug store. Ginger and Garlic oils were obtained from local market of Lahore, Pakistan. Doubly Distilled water was used for solution preparation and washing throughout the studies.

2.2. Method

2.2.1. Preparation of nano-emulsions by ultrasonic cavitation

0.1 % (w/v) garlic and ginger essential oils solutions were prepared in ethylene glycol while Neomycin sulphate (NS) solutions (0.001 %, 0.005 %, 0.01 % w/v) were prepared in doubly distilled water.

For the preparation of nano-emulsions, NS solution (100 ml) was sonicated with 2–3 drops of surfactant for 30 min at room temperature followed by dropwise addition of 10 ml of essential oil solution (using 1 ml insulin syringe) over a period of 2 h using fixed feed rate i.e., 1 drop per 30 *sec*. The temperature was maintained between 20 and 25 °C, during addition, in order to avoid degradation of essential oil as well as drug. Similar procedure was adopted for all the concentrations prepared which are labelled as defined in Table 1. The prepared nano-emulsions were kept in sealed bottle at ± 4 °C for further use and their stabilities were analyzed for a period of three months.

2.2.2. Characterization

The prepared herbal nano-emulsions (NE) were characterized by using different techniques. UV–vis spectroscopy (Analytikjena, SPECORD plus) and FT-IR (Thermo-Nicolet 6700P FT-IR Spectrometer USA) equipped with ATR was used to get insight into the NE transmittance and functional groups, respectively. HPLC-UV (Shimadzu LC-10AD) was employed to determine neomycin stability in prepared nano-emulsions by comparing the retention time with standard neomycin sulphate. The droplet size (DS), zetapotential (ZP) and polydispersity index (PDI) were determined by using Dynamic Light Scattering (Zetasizer Ver. 7.10, Serial Number: MAL1104540) at temperature 25 °C. The data was recorded using count rate of 341.3kcps and clear disposable zeta cell. Viscosity and refractive index of NE were determined without dilution using Ostwald viscometer (Fisher scientific, SI Analytics™ 285404014) and refractometer (Sigma Instruments, LI- AREF-186) after maintaining the sample temperature at 25 °C.

2.2.3. Physiochemical properties

Physiochemical properties of the prepared NE were determined by using standard procedures and results are reported as average of three readings. Refractive index was measured directly (without

Table 1
Coding of different nano-emulsions prepared with final concentration of o/w ratio.

Code	Garlic Oil (%)	Ginger oil (%)	NS (%)	Final ratio (Oil: NS)
NE-1	0.1	–	0.001	100:1
NE-2	0.1	–	0.005	20:1
NE-3	0.1	–	0.01	10:1
NE-4	–	0.1	0.001	100:1
NE-5	–	0.1	0.005	20:1
NE-6	–	0.1	0.01	10:1
NE-7	0.05	0.05	0.001	100:1
NE-8	0.05	0.05	0.005	20:1
NE-9	0.05	0.05	0.01	10:1

dilution) of all the NE and NS-1 using an Abbes type refractometer (Sigma Instruments, LI- AREF-186) at 25 ± 1 °C (Tubesha et al. 2013). Transmittance (%), of the NE and NS was determined by using UV–vis spectrophotometer (Analytikjena, SPECORD plus), after preparing 1 % solution in distilled water and taking absorbance at 250 nm. Viscosity and Density of undiluted NE and NS-1 was determined using Ostwald viscometer (Fisher scientific, SI Analytics™ 285404014) and pycnometer while maintaining temperature at 25 ± 1 °C.

2.2.4. Stability studies

The physical stability of NE was evaluated by visual inspection for checking separation of oil and any precipitation. The data for physical parameters i.e., transmittance, density, viscosity and refractive index were also acquired after preparation of NE as well as after 3 months.

To determine the stability of NS in NE, HPLC-UV was also carried by acquiring chromatograms at 250 nm by injecting 20 μL sample and using 0.1 % phosphoric acid-based Water: Methanol (50:50) mobile phase introduced at flow rate of 1.0 ml/min. C18 Column (250 mm × 4.6 mm) was used and oven temperature was maintained at 30 °C. The peak retention time as well as peak area results were evaluated by analyzing NE after preparation as well as after 3 months and expressing results as RSD with acceptance limit of 5 %.

Thermodynamic stability of the nano-emulsions was checked by performing three sets of experiments: 1) Freeze-thaw studies: The samples were kept at −20 °C for 24 h in freezer and then moved to room temperature 2) Centrifuge studies: The NE were subjected to high-speed centrifuge for 5 min at 4000 rpm 3) Heat-cool studies: The sample were kept at −4 °C for 24 h and moved to room temperature (Ali et al. 2014). Each study was carried out for two cycles and after that the NE were checked for phase separation, precipitation and physical attributes (Refractive index, transmittance, density and viscosity) to confirm stability.

2.2.5. Antioxidant studies

Antioxidant activity was determined by adopting spectrophotometric method (Brand-Williams et al. 1995). Nano-emulsions solutions (20 μg/ml) were prepared by dispersing them in ethanol, solution of ascorbic acid in same concentration was prepared and used as control. 3 ml DPPH (2,2-dipheny1-1-picrylhydrazyl) solution (0.06 g/ 150 ml ethanol) was added to test tubes followed by addition of 0.1 ml of the prepared NE solutions and NS. The test tubes were allowed to stand in dark for 30 min and after that change in color was detected by using UV/Vis spectrophotometer at 517 nm.

Following formula (Eq (1)) was applied to calculate percentage scavenging activity (SA):

$$SA\,(\%) = \frac{A_C - A_S}{A_C} \times 100 \quad (1)$$

Where, A_C and A_S is the activity of DPPH and sample, respectively.

2.2.6. Antimicrobial studies (in-vitro)

Agar-well diffusion method is used to screen the antimicrobial activity of the samples against *Bacillus spizizenii* (ATCC 6633), *Staph. aureus* (ATCC 25923), *E. coli* (ATCC 8739) and *Salmonella enterica* (ATCC 14028). The agar plate surface is inoculated by spreading 1 ml of freshly pre-cultured bacterial strain. The hole of approximately 6 mm diameter was made with help of sterile cork-borer. To this 100 μL of each nano-emulsion was introduced followed by the plate's incubation for over a period of 24 h at 37 °C. Neomycin sulphate (0.01 % solution in ethylene glycol, NS-1) is used as positive control (standard). In addition to that antimicrobial activity of NS ointment, (neomycin sulphate, 0.5 % w/w, NS-2) was also assessed by taking 0.2 g cream in ethylene glycol. All the studies were performed in triplicate and results are reported as average of data.

2.2.7. Wound healing study

An excision wound model is used to evaluate the activity of wound healing in albino rabbits. Various *in vivo* experiments are conducted in line with ethical rules of the "Animal Care & Use Committee" of the Scientific & Industrial Research Council of Pakistan (PCSIR).

Experimental animals: The study was performed on the healthy rabbits (∼1.5Kg) both male and female procured from animal facility of PCSIR Labs Complex, Lahore. The animals 15–16-week-old were housed in polypropylene cages, and divided in 12 group of 3 rabbits each, each group was kept in a separate cage, with free access to standard laboratory diet and water *ad libitum*. The rabbits were allowed to acclimatized in the animal room for a week prior to initiation of experiments.

Wound excision: All the animals were anaesthetized before wound excision. The back hair of rabbits was shaved 24 h before the test, covering an area of roughly 5 × 5 cm^2. The cleaned area of animal was anesthetized by using 25 % lignocaine followed by wound (3 × 3 cm^2) excision using sharp freshly disinfected (70 % ethyl alcohol) surgical blade. The care was taken to avoid incision of deep wound. The perpendicular cut was made on the shaved skin. The wound was allowed to remain open for the entire period of study.

Wound healing study: The group of animals were subjected to topical treatment involving application of 1 ml of each NE and 0.2 g of standard NS ointment on daily basis until complete wound healing results. The wound enclosure process (development of erythema and edema) was observed daily till wound got completely healed that almost took 2–3 weeks. The day of the injury is deemed as Day 1. The groups of rabbits were labelled as:

Group 1: Normal rats (no wound); Group 2: Control animals (only wound no treatment); Group 3: NE-1 treated; Group 4: NE-2 treated; Group 5: NE-3 treated; Group 6: NE-4 treated; Group 7: NE-5 treated; Group 8: NE-6 treated; Group 9: NE-7 treated; Group 10: NE-8 treated; Group 11: NE-9 treated; Group 12: Standard Neomycin ointment treated wound.

The wound healing (WH) or contraction rate (%) was determined by using Equation (2):

$$WH(\%) = \frac{WS_i - WS_d}{WS_i} \times 100 \quad (2)$$

Whereas, WS_i is the initial day wound on day 0 taken as 100 % while the WS_d is the wound size at specific day the measurement was done.

2.2.8. Statistical analysis

Relative standard deviation is employed on the data of HPLC and physical parameters. Correlation analysis was performed using Excell Data analysis tool to study the relationship between different variables.

3. Results and discussions

3.1. Preparation of nano-emulsions

The NEs were prepared by using garlic and ginger oils independently as well as in combinations (Table 1) by using Tween 80 as surfactant and stabilizer. Tween-80 is a non-ionic surfactant which is generally regarded as safe owing to its non-toxic and non-irritant properties (Sholihat et al. 2020). In addition to that the surfactant is also known to exhibit good dispersity and better emulsion stability (Choi et al. 2009).

FT-IR analysis of all the NE is presented in Fig. 1. The NE-1 to NE-9 spectra were acquired and compared with NS-1 to determine the impact and interaction of oil loading onto NS. The typical spectra of NS with characteristic broad band of –OH and sharp peak of C=O appears at 3352 cm^{-1} and 1635 cm^{-1}, respectively as evidenced in Fig. 1 which is in line with previous studies (Hwang et al. 2014). The broad peak of O—H is also attributed to water base emulsion as well. The similar peaks appeared in all the NE with no appreciable shift in prepared NE, which affirmed that there is no chemical interaction between NS and oils. The ginger oil, as per literature, typically show characteristic peaks of O—H stretch (broad, strong) and C—H asymmetric stretch around 3000–2900 cm^{-1}, C=O stretch at 1640 cm^{-1}, 1377 owing to CH_3 bond stretch and asymmetric angular deformation of = CH_2. In addition to that some smaller peaks around 1250–1100 cm^{-1} are attributed to = CH_2 of vinyl group (Divya et al. 2017; Farshbaf-Sadigh et al. 2021; Kalhoro et al. 2022; Visani et al. 2017) which is in good agreement with current studies. The intensities of peak appear in the spectra are small considering the lower concentrations of oil and NS used.

HPLC-UV technique was employed for assessing the stability of NS in the nano-emulsion and to rule out the possible degradation of NS owing to chemical changes in the nano-emulsion posed due to presence of other ingredients. HPLC-UV spectra of all the NE along with the standard are presented in Fig. 2. The results showed appearance of strong peak of NS at around 2.92 min in standard (Fig. 2a) as well as in all NEs (Fig. 2b-j), there were few

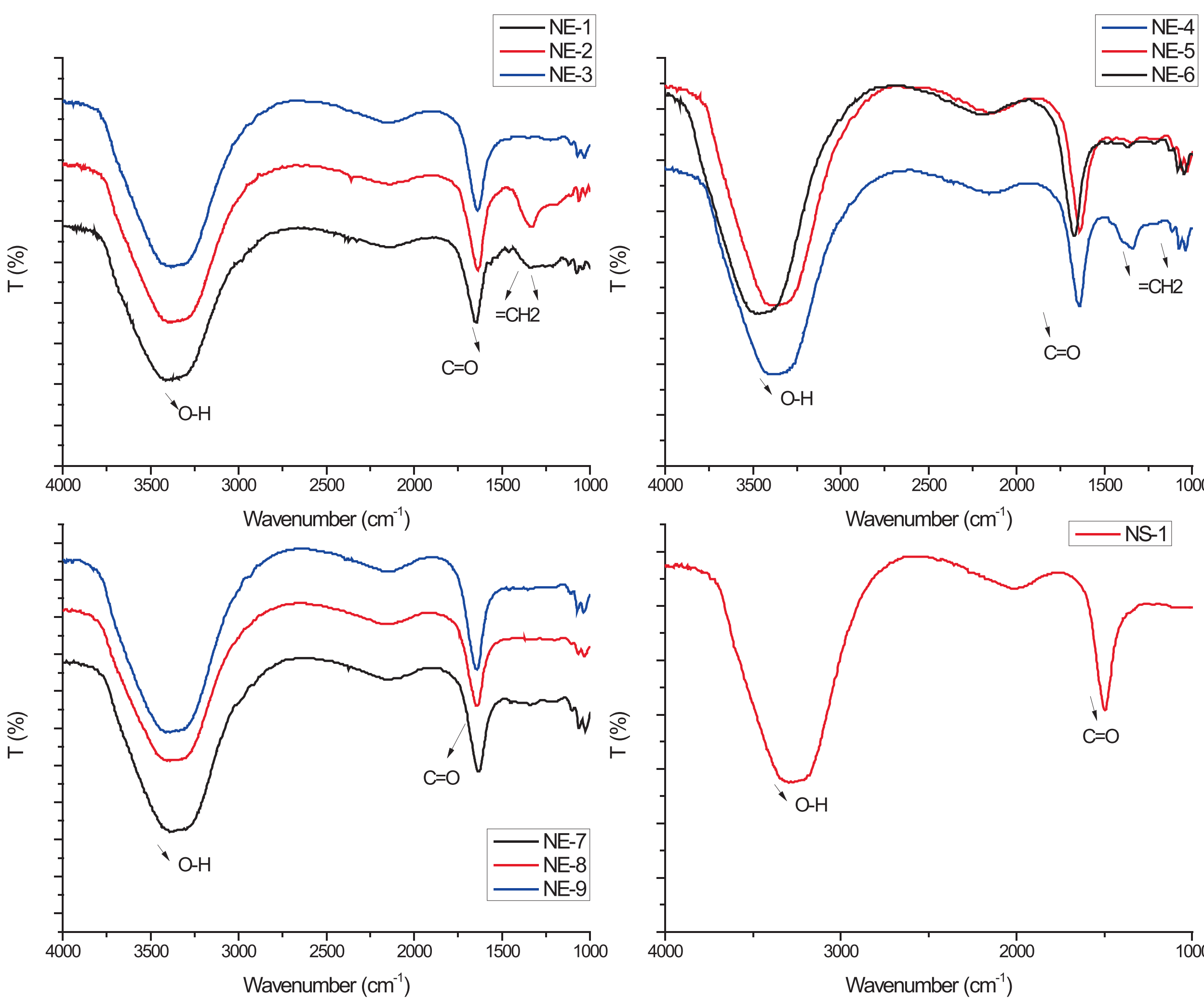

Fig. 1. FT-IR spectra of prepared nano-emulsions and neomycin sulphate.

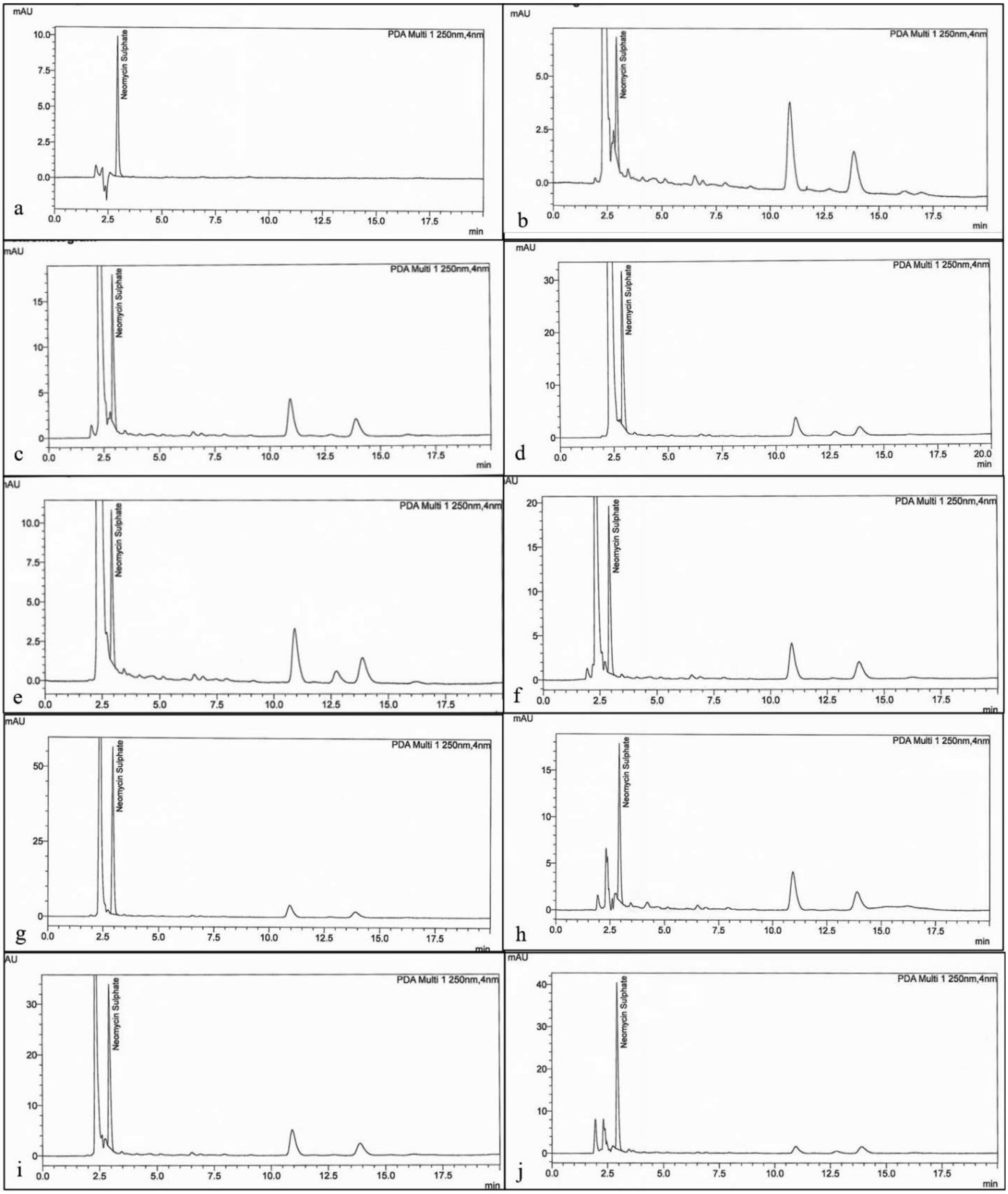

Fig. 2. HPLC-UV of a) Neomycin sulphate standard, b) NE-1, c) NE-2, d) NE-3, e) NE-4, f) NE-5, g) NE-6, h) NE-7, i) NE-8 and j) NE-9.

other peaks recorded which might be attributed to Ethylene glycol or oils. In all the samples no sign of sample degradation is recorded owing to interaction with oils.

3.2. Characterization of Nano-emulsions

3.2.1. Droplet size, zeta potential and polydispersity index

The prepared NE were analyzed for droplet size (DS), zeta potential (ZP) and polydispersity index (PDI) as these are important parameters to determine the stability of NE (Laxmi et al. 2015); the resulting data is presented in Table 2. The droplet size (hydrodynamic size) of garlic, ginger and mix oils' NE falls between 156 and 298 nm, 167 – 304 nm and 145 – 232 nm, respectively All the NE showed low PDI value (<0.5) indicating uniformity of droplet size (Ali et al. 2014; Machado et al. 1998; Rinaldi et al. 2017). Lower value of droplet size indicated the formation of well dispersed emulsion and enhanced stability by preventing adhesion and aggregation (Zhang et al. 2017).

Table 2
Physiochemical characteristics of NE.

NE	DS (nm)	ZP (mV)	PDI	RI	RI_S	RSD_{RI} (%)	T (%)	T_S (%)	RSD_T (%)	V (cP)	V_S (cP)	RSD_V (%)	D (g/cm^3)	D_S (g/cm^3)	RSD_D (%)
NE-1	156.4	0.669	0.142	1.341	1.342	0.05	96.16	96.14	0.01	1.10	1.11	0.64	1.025	1.026	0.07
NE-2	297.9	1.57	0.338	1.344	1.344	0.00	96.38	96.37	0.01	1.20	1.22	1.17	1.031	1.033	0.14
NE-3	175.1	0.931	0.240	1.341	1.342	0.05	97.95	97.95	0.00	1.23	1.25	1.14	1.038	1.041	0.20
NE-4	304.1	−3.0	0.349	1.343	1.344	0.05	96.15	96.17	0.01	1.11	1.07	2.59	1.023	1.021	0.14
NE-5	179.0	0.437	0.097	1.342	1.342	0.00	97.95	97.92	0.02	1.11	1.10	0.64	1.025	1.022	0.21
NE-6	167.0	0.766	0.212	1.341	1.342	0.05	96.61	96.64	0.02	1.20	1.21	0.59	1.032	1.034	0.14
NE-7	164.7	0.334	0.155	1.343	1.344	0.05	96.38	96.42	0.03	1.11	1.12	0.63	1.026	1.027	0.07
NE-8	232.3	1.23	0.105	1.340	1.340	0.00	96.38	96.39	0.01	1.11	1.15	2.50	1.027	1.030	0.21
NE-9	145.3	0.392	0.333	1.341	1.342	0.05	98.85	98.83	0.01	1.20	1.17	1.79	1.035	1.031	0.27
NS-1	678.0	4.34	0.494	1.331	1.333	0.11	98.17	98.19	0.01	1.00	1.01	0.70	1.022	1.025	0.21

NE = Nano-emulsion; DS = Droplet size; ZP = Zetapotential; PDI = Polydispersity index; T = Transmittance; T_S = transmittance of samples after 3 months; V = Viscosity; V_S = Viscosity of samples after 3 months; D = Density; D_S = Density of samples after three months; RSD = Relative Standard Deviation.

ZP of NE is quite variable, falling in range of 0.699 to 1.57, 0.766 to −3.0 and 0.334 – 1.23 mV for NE based on garlic, ginger and mixed oils. No significant relation is observed between the amount of drug and ZP (Table 2). ZP gives information about the charges on particles and degree of repulsion among the different particles and hence consequently stability of the particles in NE (Laxmi et al. 2015; Tubesha et al. 2013). The smaller ZP values have also been attributed to the use of non-ionic surfactants as also affirmed by previous studies (Ahmad et al. 2019a).

The correlation analysis showed positive correlation between ZP and DS (r^2 = 0.99) and PDI (r^2 = 0.97) for the NE of ginger, while for garlic NE the negative correlation is observed for ZP and DS (r^2 = -0.99), and PDI (r^2 = -0.75). In case of mixture of oils, the positive correlation is observed between ZP and DS (r^2 = 0.91) while negative correlation is observed between ZP and PDI (r^2 = -0.61).

3.2.2. Refractive index (RI) and transmittance (T)

RI is an important property of NE signifying its isotropic nature and chemical interaction between the drug and other ingredients of NE. Values of all the NEs were found in range of 1.340 – 1.344 indicating their isotropic nature. Furthermore, the similarity among RI values and their small values points to uniformity and clarity of NE, respectively (Laxmi et al. 2015; Sondari and Tursiloadi 2018; Su et al. 2017). Clarity of the NE, is also determined by acquiring (T%). Table 2 defines the values as obtained for the NE samples; the values of T approaching to 100 % are sign of clear and transparent NE (Laxmi et al. 2015).

3.2.3. Viscosity and density

Viscosity of NE is also indicative of its stability (Laxmi et al. 2015). Table 2 presents the values of NE which fall in range of 1.10 – 1.23cP which indicated very dilute solution. The lower viscosity values points to formation of O/W NE. The viscosity of NE is comparatively greater than NS-1, owing to presence of oils. As the concentration of NS increase, there is small increase in the viscosity of NE. As reported by earlier studies, the low viscosity values are typical of NE (Ali et al. 2014; Kotta et al. 2015). Similarly, in case of density, increase in concentration results in increasing density. Highest value of density was observed for NE-3 (1.038 g/cm^3) and lowest was noticed for NE-4 (1.023 g/cm^3). The positive relationship was also affirmed from the correlation studies carried out between concentration of NS and density and viscosity. For ginger, garlic and mixture of oils the correlation coefficient (r^2) values obtained for density and viscosity are as follows: 1.0, 0.97, 0.94 and 0.93, 0.90, 0.90 respectively.

3.3. Stability studies

Stability study data (Table 2) was acquired by determining physiochemical parameters i.e., Refractive index (RI_S), Transmittance (T_S), Viscosity (V_S) and Density (D_S) of the prepared NE after 3 months. The data was evaluated for significant change by RSD values which for all the parameters fall in acceptable range i.e. (<5%). Table 2 shows that there is no significant variation among the data recorded for all NE after 3 months confirming stability of NE.

In addition to that one sample from each lot (i.e., NE-1 (garlic), NE-4 (ginger) and NE-7 (garlic + ginger)) was analyzed after three months to check the degradation of the drug under storage conditions and chromatograms showed that there was no significant change in NS peak intensity (i.e., 5 % RSD) from the one recorded earlier (data not presented). Hence, confirming the stability of NE over a period of three months.

The thermodynamic stability studies were also conducted for three sets of parameters, the results are presented in Table S2. The physical inspection of the samples showed no physical change (phase separation) (Fig. S1) after employing the freeze–thaw (FT), centrifuge (C) and heat-cool (HC) cycles. In addition to that the RI, density, viscosity and transmittance data procured after performing set of experiments showed no significant change in the values (RSD < 5 %) which confirms that nano-emulsions are thermodynamically stable as well (Ali et al. 2014; Laxmi et al. 2015; McClements and Jafari 2018).

3.4. Antimicrobial and antioxidant activities

Antimicrobial activity of NE and the standard was acquired against both gram positive (*B. spizizenii & Staph. aureus*) and negative (*E. coli, S. enterica*) bacteria (Fig. 3). The NS-1 was employed as positive control. In general, NE-2 (garlic) have highest antimicrobial activity against all microbes except *Staph. aureus* in which case NE-4 (ginger) performed better. The order of activity observed for different microbes is as follows:

B. spizizenii: NE-7 < NE-1 < NE-5 ~ NS-1 < NE-9 < NE-3 < NE-6 < NE-4 < NE-8 < NE-2.

Staph. aureus: NE-6 < NE-9 < NE-1 < NE-7 ~ NS-1 < NE-8 ~ NE-5 < NE-3 < NE-2 < NE-4.

E. coli: NE-7 < NE-1 ~ NS-1 < NE-6 < NE-5 < NE-9 < NE-3 < NE-8 < NE-4 < NE-2.

S. enterica: NE-7 < NE-1 < NE-3 ~ NS-1 < NE-9 < NE-8 < NE-6 < NE-5 < NE-4 < NE-2.

The NE of mixture of garlic, ginger and NS doesn't enhance the activity of each other rather showed reduced antimicrobial activity as compared to garlic-NS and ginger-NS nano-emulsions. This can be attributed to antagonistic effects of garlic and ginger in cumulative setup. Most of the NE showed improved activity as compared to the NS-1 which depicts the higher efficacy of these NE for suppressing the growth of microbes and hence inflammation.

NS-2 (neomycin ointment) didn't show any Zone of inhibition (ZOI) (Fig. 3a); hence the data is not depicted in Fig. 3b which confirms the resistance of selected microbes against the drug.

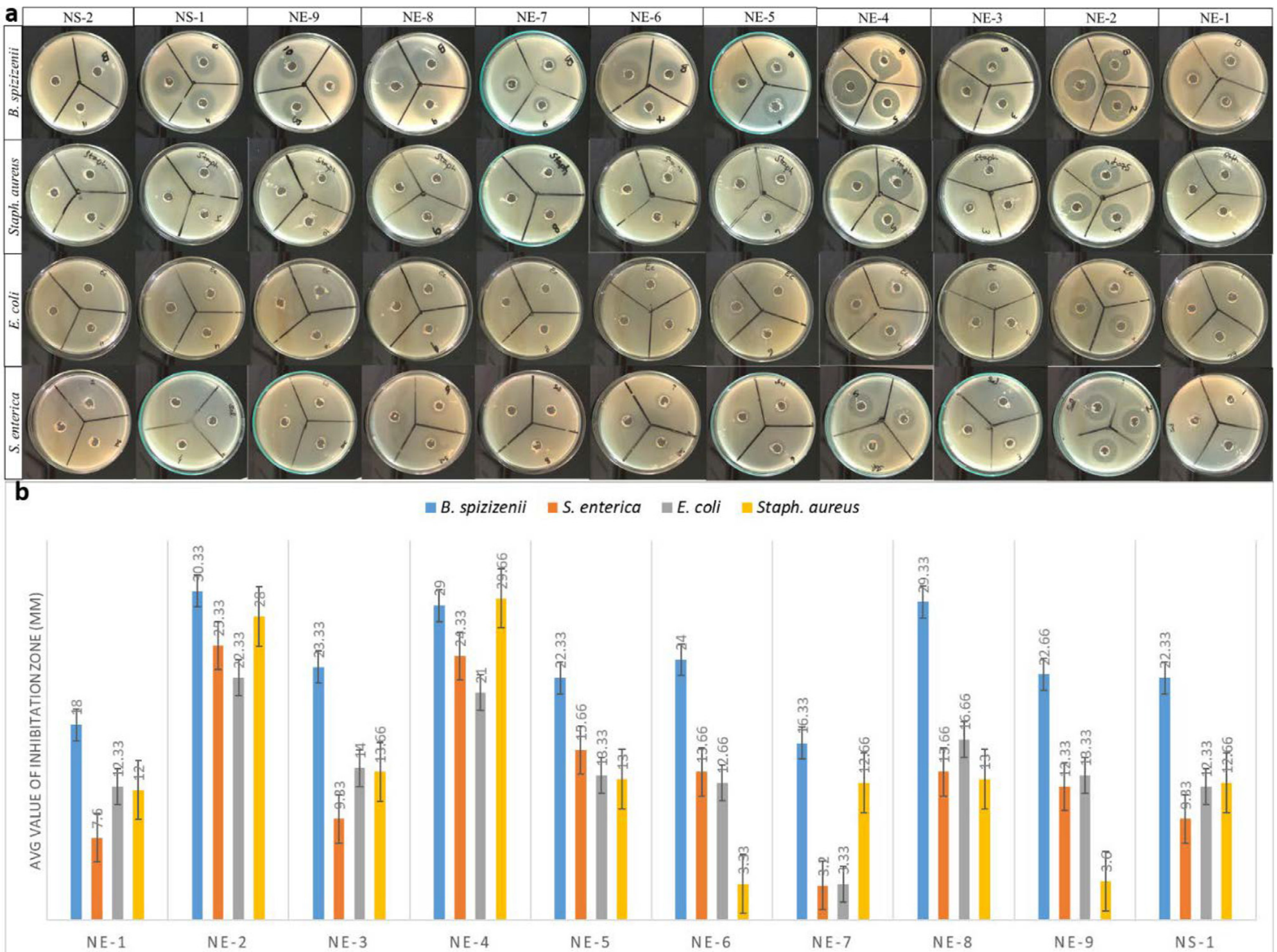

Fig. 3. Antimicrobial activity of NE and NS a) Zone of inhibition as observed and b) Graphical representation of zone of inhibition.

Bacillus sp., *S. aureus* and *E. coli* are one of the known culprits of Cutaneous infections. All of these microbes in one way or other can cause severe wound infections and even can lead to hospitalization in patients suffering from hematological malignancies and immunodeficiency disease. Over the years these microbes have developed resistance against many antibiotics (Gordon et al. 2008; Machado et al. 1998; Mandal et al. 2010; Petkovsek et al. 2009; Sunder et al. 2012) as can also be seen in this study where ointment (NS-2) didn't show any appreciable impact on the microbial strains under study.

The antioxidant studies of the prepared nano-emulsions were performed and results are presented in Fig. 3S. Almost all the NE shows similar antioxidant potential i.e., < 99 % which can be attributed to the similar concentration of the essential oils used in the study. The high antioxidant potential of NE is in line with previous studies. The antioxidant activity plays crucial role in wound healing activity especially by tackling inflammation (Abdellatif et al. 2021; Ahmad et al. 2019a; Ahmad et al. 2019b).

3.5. Wound healing activity

Wound healing images and percentage wound area contraction of the control group and groups with NEs application and positive control group applied with marketed preparation of antibiotic Neomycin, NS-2 are presented in Fig. 4. The wound contraction data was procured till Day 9 and wound healing was measured (Fig. 5). All the NEs as compared to NS-2, significantly enhanced wound contraction resulting in 100 % wound healing in case of NE-3, NE-6, NE-8 and NE-9 while for NE-1, NE-2, NE-4, NE-5 and NE-7 the wound area contraction is observed to be 86–89 %. In contrast NS-2 resulted only in 71 % wound contraction. Improved wound healing potential of NEs as compared to NS was attributed to nanosized particles that enable enhanced penetration. Furthermore, the additive action of NS with oils is found to be much more improved as compared to NS alone when applied in same ratio. The complete epithelization and skin hair covering occurred within 9 days in case of NE. As the wound healing process is often accompanied by inflammation, but in all the NE the wound recovery observed is fast with no aggravated inflammation. On Day 3, there was significant wound recovery in case of NE-3, NE-6 and NE-8. Apparently, the introduction of garlic and ginger performed better than alone garlic or ginger and with optimized concentration of 0.01 % of NS.

The earlier studies, also indicated the higher activity of the oils as compared to the NS (Sugumar et al. 2014). The current study has the advantage of promoting fast epithelization in addition to enhanced wound healing (9 days) as compared to the other oil based nano-emulsions (Table 1S) like Eucalyptus oil (24 days) (Alam et al. 2018), clove oil (24 days) (Alam et al. 2017), curcumin (24 days) (Ahmad et al. 2019a), lavender and licorice (16 days) (Kazemi et al. 2020). This increased healing is also affiliated with antimicrobial activity of the prepared NE leading to early healing (Abdellatif et al. 2021) as well as improved proliferation of epithelial cells (Alam et al. 2017).

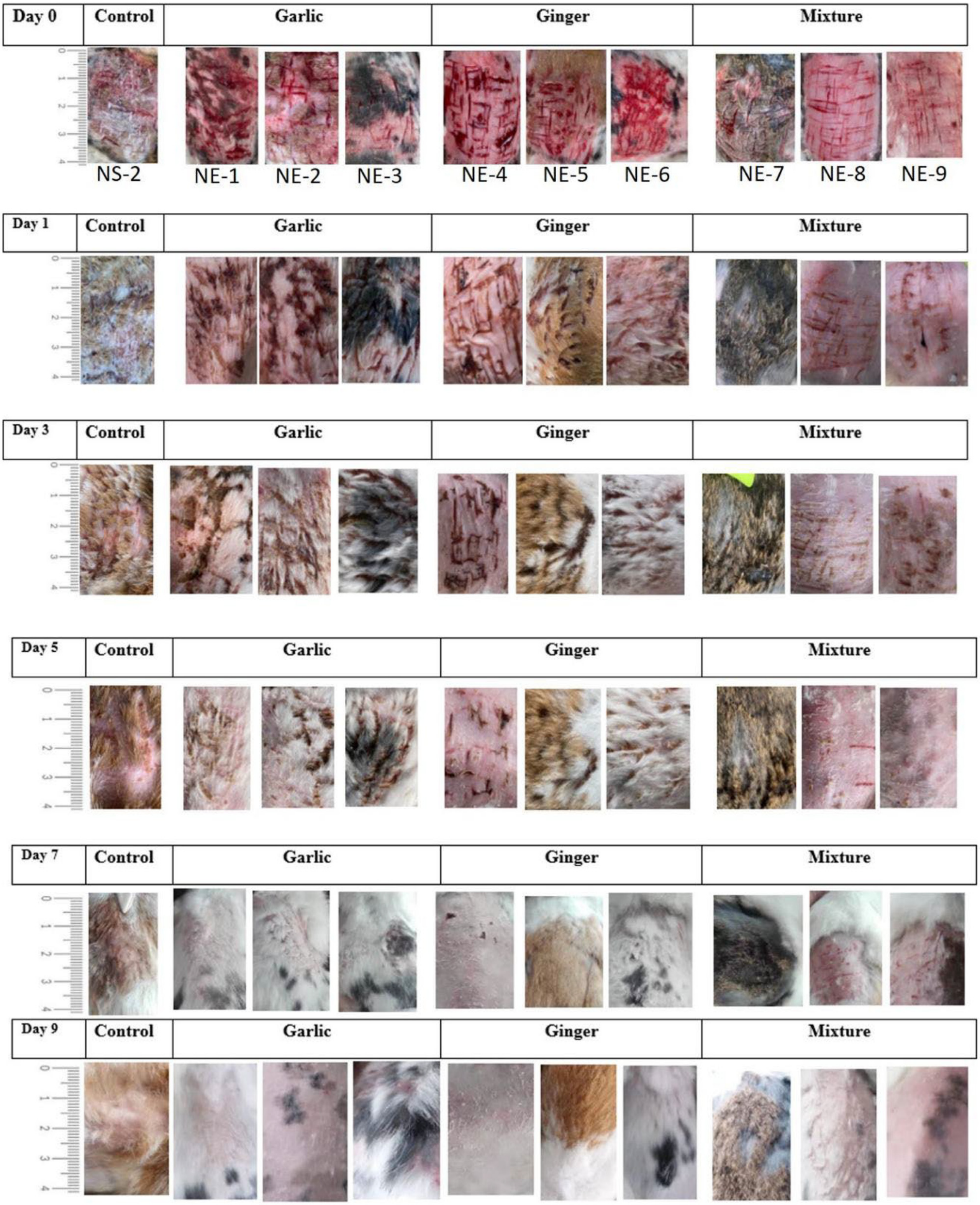

Fig. 4. Wound healing activity of prepared NE and NS in rabbits as a function of time.

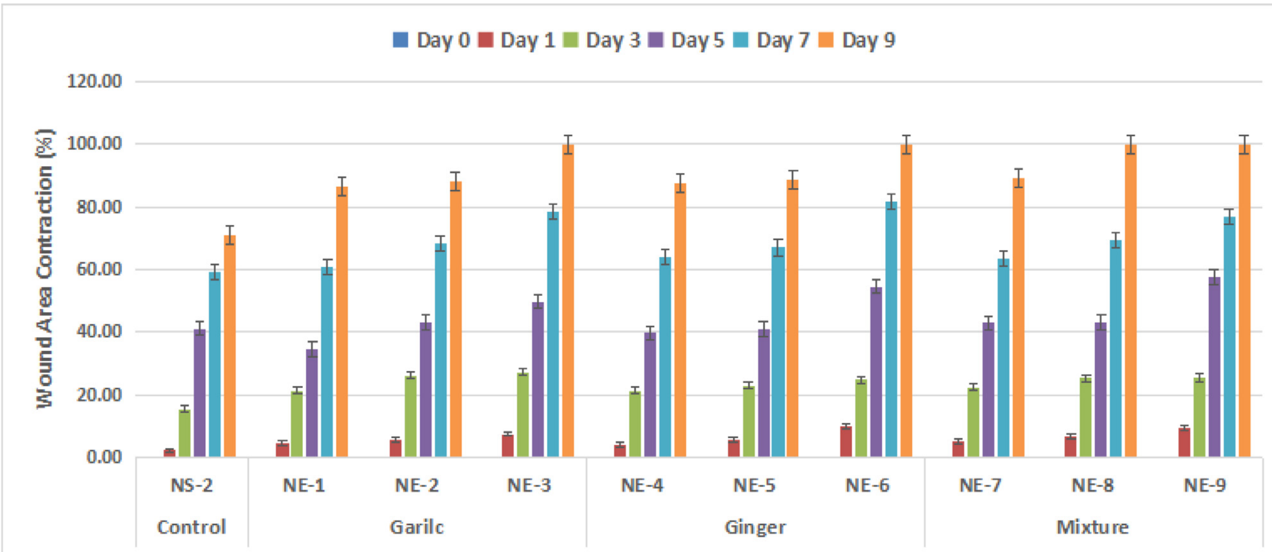

Fig. 5. Graphical representation of comparative wound healing activities of prepared NEs and NS in rabbits.

4. Conclusion

In the present study, nine ginger and garlic oils-based neomycin sulphate nano-emulsions were prepared by using ultrasonic approach. Total of nine nano-emulsions were prepared resulted have the size in range of 145–304 nm and found stable over a period of three months. The thermodynamic stability studies performed by employing freeze–thaw cycle, centrifuge cycle and heat-cool cycle affirmed the high stability of these nano-emulsions. Furthermore, the nano-emulsions are found stable over the period of three months without showing any significant change in physical attributes and phase separation. The nano-emulsions showed excellent antimicrobial activity against both gram positive and negative bacterial strains in contrast to neomycin sulphate used as positive control. Furthermore, the NEs accelerated the wound healing stages as compared to the Neomycin sulphate ointment leading to complete healing of wound in just 9 days, suggesting the great potential of these nano-emulsions for wound healing formulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2022.09.015.

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