A DISSERTATION ON

Development of a UV-protective and wound healing nanoformulation for pharmaceutical applications

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF TECHNOLOGY
IN BIOTECHNOLOGY

BY
Anjeeta Singh
M. Tech Biotechnology (IV Semester)
Roll No: 2001361003

UNDER THE SUPERVISION OF
Dr. Iffat Zareen Ahmad
Professor
Department of Bioengineering

INTEGRAL UNIVERSITY, DASAULI, KURSI ROAD
LUCKNOW- 226026

DECLARATION FORM

I, **Anjeeta Singh**, a student of **M.Tech Biotechnology** (2^{nd} Year/ 4^{th} Semester), Integral University have completed my six months dissertation work entitled **"Development of a UV-protective and wound healing nanoformulation for pharamaceutical applications"** successfully from **Integral University** under the able guidance of **Dr. Iffat Zareen Ahmad.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Anjeeta Singh

Date

Dr. Salman Akhtar

Course Coordinator

Date

INTEGRAL UNIVERSITY

Established Under the Integral University Act 2004 (U.P. Act No.9 of 2004)
Approved by University Grant Commission
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

CERTIFICATE BY SUPERVISOR

It is hereby certified that **Ms. Anjeeta Singh** (Enrollment Number 2000102269) has carried out the research work presented in this thesis entitled **"Development of a UV-protective and wound healing nanoformulation for pharmaceutical applications"** for the award of **M. Tech Biotechnology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M. Tech Biotechnology**.

I wish her good luck and bright future.

Dr. Iffat Zareen Ahmad
Professor
Department of Bioengineering

INTEGRAL UNIVERSITY

Established Under the Integral University Act 2004 (U.P. Act No.9 of 2004)
Approved by University Grant Commission
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Anjeeta Singh,** a student of **M. Tech Biotechnology** (2nd Year/ 4th Semester), Integral University has completed her six months dissertation work entitled **"Development of a UV-protective and wound healing nanoformulation for pharmaceutical applications"** successfully. She has completed this work from Integral University under the guidance of Dr. Iffat Zareen Ahmad, Professor. The dissertation was a compulsory part of her **M. Tech Biotechnology .**

I wish her good luck and bright future.

Dr. Snober S. Mir

Associate Professor

Head Department of Biosciences

Integral University, Lucknow

INTEGRAL UNIVERSITY

Established Under the Integral University Act 2004 (U.P. Act No.9 of 2004)
Approved by University Grant Commission
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Anjeeta Singh,** a student of **M. Tech Biotechnology** (2nd Year/ 4th Semester), Integral University has completed her six months dissertation work entitled **"Development of a UV-protective and wound healing nanoformulation for pharmaceutical applications"** successfully. She has completed this work from Integral University, Lucknow under the guidance of Dr. Iffat Zareen Ahmad. The dissertation was a compulsory part of her **M.Tech Biotechnology.**

I wish her good luck and bright future.

Dr. Alvina Farooqui
Head
Department of Bioengineering
Faculty of Engineering

ACKNOWLEDGEMENT

First of all, I bow in reverence to the Almighty for blessing me with strong will power, patience and confidence, which helped me in completing the present work.

At the very outset I pay my warm thanks to our Honorable **Chancellor and Founder, Integral University, Lucknow. Prof. S.W Akhtar** for providing excellent infrastructure and Lab facilities at IIRC-7 Integral University, Lucknow. I am also grateful to Honorable **Vice Chancellor Integral University, Lucknow. Prof. Javed Musarrat** for his continuous motivation and a Special vote of thanks to **Pro chancellor, Dr. Nadeem Akhtar** for his encouragement and support.

I would like to express my special thanks to **Dr. (Er.) Alvina farooqui (Head, Department of Bioengineering)** for given me an opportunity to join the department laboratory and providing all the necessary facilities ever since I started my work.

I would I like to express my deep sense of gratitude to my Supervisor **Dr. Iffat Zareen Ahmad (Professor),** Internal advisor **Dr. Snober S. Mir (Associate Professor)**, and Course coordinator **Dr. Salman Akhtar (Associate Professor)** for their invaluable guidance throughout the course of my dissertation work and academic session. It would have been impossible to complete this work in so short a time without her constant guidance.

I would like to thank respected dissertation coordinators **Dr. Roohi, Dr. Owais Yousuf, Er. Khwaja Osama** for their continuous support and help.

My acknowledgement will be incomplete if I do not mention **my parents and my family** with whose blessing; I was able to achieve my goal successfully. There are no words to express my feelings toward them. I silently acknowledge my debt to them.

Date: **ANJEETA SINGH**

LIST OF ABBREVATIONS

Abbrevation	Full form
COM	*Cydonia oblonga*Miller
SCY	Scytonemin
UV	Ultravoilet
MAA	Mycosporine-like amino acids
SOS	Save our souls
ROS	Reactive oxygen species
SOD	Superoxide dismutase
ZnO	Zinc oxide
PEO	Plasma electrolyte oxidation
QMC	Quince seed mucilage
HW	Hot water
HexB	Hexosaminidase B
RBL	Rat basophilic leukaemia
IgE	Immunoglobulin E
XO	Xanthine oxidase
NO	Nitrogen monooxide
VH	Vascular hypertrophy
C6H18O9	Chlorogenic acid
RBC	Red blood cells
UC	Ulcerative colitis
UV	Ulcer index value
LPL	Lipoprotein lipase
NE	Nanoemulsion
ME	Microemulsion
GPS	Gastroesophageal reflux
FBS	Fetal bovine serum

EDTA	Ethylenediamine tetraacetic acid
DMEM	Dulbecco’s modified eagle medium
HaCaTs	Cultured human keratinocyte (cells)
PDI	Poly dispersity index
DLC	Diamond-like carbon

CONTENTS

LIST OF FIGURES

13	Scytonemin–*Cydonia oblonga* nanoemulsion increased keratinocyte migration and proliferation. Representative images of the transwell migration assay upon treatment of keratinocytes with *Scytonemin-Cydonia oblonga* extract and its loded nanoemulsion (magnification × 20).	40
14	Migration ability of keratinocytes represented as the percentage of cells penetrating the membrane.	41

LIST OF TABLES

1. INTRODUCTION

Cydonia oblonga Mill. belongs to the Rosaceae family and is a medicinal plant that is used to treat a variety of ailments such as cancer, diabetes, hepatitis, ulcers, respiratory and urinary infections, etc. Quince is high in secondary metabolites such as phenolics, steroids, flavonoids, terpenoids, tannins, sugars, organic acids, and glycosides. Different parts of *C. oblonga* (CO) have been linked to a wide range of pharmacological effects, including antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, cardiovascular, antidepressant, antidiarrheal, hypolipidemic, diuretic, and hypoglycemic. Glucuronoxylan, a polysaccharide mucilage extruded from the quince seeds, is used in dermal patches to promote healing (Karimi *et al*., 2017).

Traditional treatments made from substances with a natural origin, like plant extracts, honey, and larvae, are intriguing alternatives. These treatments open up new avenues for the treatment of skin conditions while improving healthcare access and removing some of the drawbacks of contemporary products and therapies, such as their high prices, protracted manufacturing processes, and rise in bacterial resistance (Souto et al., 2011).

Due to the secondary metabolites found in plants, such as tannins, terpenoids, alkaloids, and other substances, various plant parts, including roots, flowers, fruits, leaves, and seeds, have historically been used frequently to treat a variety of complications **(**Harborne *et al*., 1999). Quince extracts have historically been used as dietary supplements as well as a form of treatment for infections and inflammatory diseases (Church *et al*., 2013). Quince seed mucilage has historically been used as a home remedy for wound healing (Sadeghi Mahoonak *et al*., 2017).

Scytonemin (SCY), a lipid-soluble, highly stable, yellow-brown secondary metabolite, is accumulated in the extracellular polysaccharide sheath of some but not all cyanobacteria in response to UV-A radiation stress (UV-A; 315-400nm), acting as a photoprotective substance. Scytonemin exhibits reasonable levels of radical scavenging activity, making it a potential natural UV sunscreen and antioxidant for skin protection (Gao *et al*., 2011). Dermatologists are very interested in this because it has an advantage over synthetic sunscreen compounds in that it has evolved through a selection process that makes it suitable for human applications.

Cyanobacteria produce the UV-protecting substances MAAs and scytonemin, which have the capacity to decrease toxicity. Cyanobacteria can repair UV-induced DNA lesions using a number of techniques, including photo, excision, recombination, and repair mechanisms. Stress tolerance in cyanobacteria is influenced by the SOS response and cell death. When grown under white light and in conjunction with UV-A and UV-B, it was noted that P. Murrayi grew linearly as UV-A levels increased and that the UV-B inhibition balanced the damage and repair processes (Quesada *et al*., 1995). Cyanobacteria are the most environmentally successful prokaryotes on the planet, and it appears that they have a stress tolerance mechanism.

In addition to being UV protective, cytotoxic, antibacterial, antitumor, antiviral, antifungal, antimalarial, anti-inflammatory, antiprotozoal, and antituberculous, cyanobacteria are also capable of producing secondary metabolites with significant bioactivity. Cyanobacteria existed on Earth billions of years ago when the atmosphere lacked oxygen. In order to protect themselves from the effects of UV radiation, cyanobacteria have evolved a number of defence mechanisms, such as the production of ultraviolet protective compounds, proteins, and antioxidants. Pharmaceutical and biotechnology firms use cyanobacterial metabolites in a variety of ways. Plant extracts, proteins, pigments, and flavonoids are commonly used in nanoparticle synthesis (Khalifa *et al*., 2021).

Long-term exposure to UV rays causes a variety of skin problems such as sunburn, wrinkles, skin ageing, hyperpigmentation, and can even lead to skin cancer. The good news is that UV radiation can be lessened by taking a few easy precautions. Some of the beauty ingredients are found in blue-green algae. Natural UV filters and moisturisers can be made from cyanobacteria because of their capacity to retain water and produce UV protective compounds (Derikvand *et al*., 2017). They are also high in antioxidants, which help to neutralise free radicals (Singh *et al*., 2004). Nanotechnology provides amazing, remarkable, and cutting-edge approaches to product delivery. Liquid lipid, solid lipid non-ionic surfactant niosomes, NE, and ME are new lipid carrier vehicles with high penetrating power, site-targeted drug delivery, high stabilisation and entrapment efficiency, and controlled and sustained drug release (Ramanunny *et al*., 2021). Due to their slower kinetics, lower cost, and environmental friendliness, nanoparticles derived from cyanobacteria have demonstrated better results (Kumar *et al*., 2019). Liposomes can be used to deliver a variety of cosmetic creams, including anti-aging, moisturising, sunscreen, and beauty products (Bilal *et al*., 2020). Moisturizers are

transported by lipid carriers and nanoemulsion systems. They have the ability to lift and rejuvenate the skin, renew collagen, and retain moisture (Fernandes et al., 2005). Solid lipid nanoparticles are thought to have been replaced by nanostructured lipid carriers (NLC) (SLN). NLC increases the hydration and penetration of the skin, maintains drug stability, and enhances UV protection with fewer side effects (Müller *et al*., 2002).

By reducing immunogenicity to infectious agents and harmful byproducts, cyanobacterial bioactive components play a significant role in the diagnosis of inflammation. In the treatment of various diseases, they function as promising anti-inflammatory agents. These compounds must be produced on a large scale (Dwivedi *et al*., 2022). The discharge of toxins from migrating cells and tissue causes inflammation. Cyanobacteria defend themselves against inflammation through a number of mechanisms: Activation of pro-inflammatory cytokines, intercellular cell adhesion molecules, NO, and nuclear factor kappa B were all inhibited by chemokine activation via cyclooxygenase-2, while antioxidant enzyme activity and peroxisome proliferator-activated receptor activity increased (Balan *et al*., 2017).

Ozone depletion has caused an increase in anthropogenic chemical levels, which has had detrimental effects on the earth's ecosystem (Bornman *et al*., 2015). Since blue-green algae are more vulnerable to ultraviolet stress, a number of UV protective mechanisms have been developed to protect cyanobacterial cells from the harmful effects of UV radiation. In response to the production of ROS, cyanobacteria produce defence and coping mechanisms by controlling gene expression in various cellular signalling pathways. They generate antioxidants like superoxide dismutase, catalase, and peroxidase, which convert reactive oxygen species (ROS) into nontoxic products. Because of ozone depletion, the level of anthropogenic chemicals has increased, causing a serious problem with adverse effects on the earth's ecosystem (Bolaji et al., 2013). Since blue-green algae are more sensitive to ultraviolet stress, various UV protective mechanisms have been developed to protect cyanobacterial cells from the harmful effects of ultraviolet radiation. Various biochemical processes are either directly or indirectly affected (Pattanaik et al., 2007). Cyanobacteria produce protective and adaptive mechanisms in response to reactive oxygen species generation by regulating gene expression in a variety of cellular signalling pathways. They produce antioxidants like superoxide dismutase (SOD), peroxidase, and catalase, which convert reactive oxygen species (ROS) into nontoxic products.UV radiation damages DNA and causes lipid peroxidation. The outer layer of exopolysaccharides, which protects cyanobacteria from

UVR, is very important. They can repair damaged DNA and protect it from lipid peroxidation by removing the ROS produced by UV radiation (Rastogi *et al*., 2014).

Nanoformulations have received a lot of attention as quickly developing materials with a variety of applications. Due to the variable nature of nanoparticles and the lack of reference materials for the standardisation of tools, sample preparation, estimation of their concentration, and data interpretation, investigating nanoparticles is a challenging task. Different methods can be used to characterise nanoparticles. Particle dispersion, distribution, shape, surface characteristics, and crystallinity were used to categorise nanoparticles. A single nanoparticle's properties can be evaluated using a variety of methods. The relationship between the structure and materials used in nanoparticles is maintained in large part by the size distribution of the particles, which can be measured using the dynamic light scattering method and electron microscopy. A beam of electrons is used in the scanning electron microscopy technique to examine a sample's surface and create an image. Similar to this, transmission electron microscopy creates an image by passing an electron beam through the sample. The image is magnified and focused onto the photographic plate after the sample section, which should be ultra thin (less than 100 nm thick), is used. The interaction between the probe and the sample generates images in atomic force microscopy. This determines a sample's mechanical properties, such as stiffness (Alonso *et al*., 2003). The Stokese Einstein equation is used to correlate the particle's size and velocity with dynamic light scattering, a general technique for analysing hydrodynamic particle size and distribution across a range of sizes. The polydispersity index represents the size of nanoparticles. The main limiting factor is dispersion, so precise size determination is required (Müller *et al*., 2002). Infrared spectroscopy (also known as vibrational spectroscopy) provides information on the absorption, emission, and reflection of infrared radiation by molecular species. It identifies functional groups in solid, liquid, and gaseous states. Different ligands attached to nanoparticles can be recognised with ease (Duerst *et al*., 2007). Circular dichorism spectroscopy is used to create hybrid nanoparticles, which are formed by aggregating nanoparticles with photosensitizer molecules. It is primarily used in organic/inorganic hybrid nanoparticles. This new creation must bring with it new challenges. The primary requirement is to identify the presence of these aggregates (Visheratina *et al*., 2019).

Wound healing is critical in restoring the skin's barrier function. It is a complicated process that has four stages: hemostasis, inflammation, proliferation, and

tissue repair. Cells in the wound bed must proliferate and migrate during healing. The rate-limiting event in wound healing is this migration phase (Gefen *et al*., 2019).

In terms of surface area, the skin is the largest organ in the body. It is a crucial component that protects inner organs from thermal shock, microbial infection, UV radiation, and mechanical harm. Normally, living things can't regenerate their tissue. There are several wound healing therapies available, but they are only partially successful. Hence, there is a requirement for more potent wound healing treatments. Multiple cell types within these three layers must cooperate at specific stages to heal a wound in the skin. As a result, healing of damaged organs requires a multi-step process that includes collagen and inflammatory cell migration, cytokine activity, extracellular matrix deposition, and scar remodelling (M.E. Gilbert *et al*., 2004). The skin is the largest organ by surface area in the human body. It is the critical structure that shields internal tissues from mechanical damage, microbial infection, ultraviolet radiation, and extreme temperature. Usually living organisms are unable to regenerate their tissue. While several therapies for wound healing are available, these are only moderately effective. Thus there is a need for more effective therapies for healing wounds. When the skin is wounded, multiple cell types within these three layers need to coordinate at precise stages to bring about healing. Therefore, restoration of injured organs involves a complex process: migration of inflammatory cells and collagen, cytokine actions, deposition of the extracellular matrix, and scar remodeling (Metcalfe *et al*., 2007). With few exceptions, a scar develops as a result of wound healing. Excessive scarring shifts the balance of hypertrophic scarring and keloid formation towards fibrotic states (Walmsley *et al*., 2015). Increasing evidence indicates that different cellular reactions to mechanical stress within the healing skin cause scarring (Duscher *et al*., 2014). In general, this process is unaffected by the sort of injury and varies little from tissue to tissue. There are a number of complex and redundant mechanisms that work in tandem to facilitate and accelerate wound healing **(**Singer *et al*., 1999). The healthcare system may be severely burdened in some situations where the skin's regenerative capabilities are suspended and wounds cannot heal physiologically. Chronic wounds are defined as wounds that do not heal within 90 days (Vasconcelos *et al*., 2011). Large burns and diabetic wounds are two examples of chronic wounds; they are difficult to treat, expensive, and at higher risk of infection, necessitating frequent surgical procedures (Zhang, *et al*., 2009). Because of the rising rates of both diabetes and obesity, the burden of treating chronic wounds on healthcare systems is increasing each

and every day (Sen, *et al*., 2009). Improper wound care causes recurrence and can lead to limb amputations and death.

2. REVIEW OF LITERATURE

In this study, an attempt is being made to develop a UV protective nanoemulsion with wound healing properties which could find suitable applications in pharmaceutical industries. The nanoemulsion might find applications in the form of cosmeceutical products to prevent scarring of wounds due to sun exposure. The *Cydonia oblonga* Mill. extract was prepared from the seeds of the plant and the scytonemin extract was prepared from the cyanobacterium, *Scytonema javanicum*.

The impact of quince seed mucilage on the proliferation of human skin fibroblasts to investigate the mechanism of wound healing (Ghafourian *et al*., 2015). Mucilage was applied to human skin line cultures at various concentrations (50, 100, 200, and 400 g/mL), and the effects of the mucilage were noticed after 12, 24, 48, and 72 hours. According to research using the microculture tetrazolium assay, quince mucilage accelerated the proliferation of human skin fibroblasts after 48 hours, even at low concentrations (50 g/mL). In a different study, mice were used to test the effectiveness of an ethanolic extract of quince seeds for treating second-degree burn wounds. The results revealed that the extract, at a concentration of 1%, produced wound healing rates of 99.5 percent compared to the industry standard of 92.97 percent for sulfadiazine. Similarly, silver nanoparticles of mucilage and methanolic and acetonic extracts of quince seeds were found to be effective against *S. aureus*-infected wounds (Khan *et al*., 2021). Applying 5, 10, and 20% Quince Seeds Mucilage Cream in Eucerin Base on the Skin Wounds of White Iranian Rabbits has also demonstrated the effectiveness of Quince Seeds Mucilage for Skin Wound Healing. In 13 days of treatment, QMC (20%) cream completely healed the wounds (Karimi *et al*., 2017). Dermal patches of quince mucilage were prepared and tested for mechanical, microstructural, antioxidant, antibacterial, physical, and thermal parameters by adding 1, 1.5, and 2 percent v/v of oregano essential oil (Cheng *et al*., 2017). Fekri *et al*., 2008 examined the water content, production, protein content, and ash content of mucilage. Protein, ash, and moisture content were discovered to be 4.38, 10.97, 20.9, and 8.24 percent (Cheng *et al*., 2017). Quince leaves have been shown to have wound-healing properties (Sabale *et al*., 2012). In a double-blind clinical trial, the effective topical wound healing containing 10% quince seed mucilage for 34 patients referred to a dermatology clinic for benign lesions to biopsy or surgery in a double-blind clinical trial (Mousavi *et al*., 2006).

The body's skin is the first line of defence against pathogens. That's why burns and skin wounds require special attention and treatment. Hence, it's crucial to treat the wound as soon as possible and appropriately. Specialized wound dressings can be made using nanotechnology to control the timing, location, and rate of drug release from wounds. Additionally, nanobandages can be applied over a wound to stop microorganisms from getting inside the body and infecting it. Using the electrospinning method and various materials such as natural and synthetic polymers, very fine and continuous fibres with diameters ranging from micrometres to several nanometers can be produced. In this study, antibacterial nanobandages were made using zinc oxide nanoparticles made using a green method (using walnut leaf extracts), quince seed mucilage (a new biopolymer), chitosan, and PEO. The best fibres with suitable diameters, shapes, and ZnO nanoparticle loading were obtained in a ratio of 20:80 quince seed mucilage to chitosan/PEO. The best electrospinning parameters were as follows: voltage = 18 kV, polymer solution feed rate of 1 mL/h, and needle-to-collector distance of 12 cm. The nanofibers produced were characterised using a tensile test. Animal tests revealed that a nanobandage containing ZnO nanoparticles had a significant treatment effect on 2 degree burn wounds, with the burn healing faster and no infections detected in the wound. After 21 days, the skin had fully recovered, and their bodies were free of any signs of burns. The study revealed that the developed nanobandage had a significantly greater impact on wound healing than the control and nanobandage without nanoparticles (Darvishi *et al*., 2021).

Secondary metabolites with significant bioactivity, such as ultraviolet defence, cytotoxicity, antibacterial, antiviral, anticancer, antimycotic, antituberculosis , antiprotozoal, anti-inflammatories, and antimalarial, are produced by cyanobacteria. Cyanobacteria lived on Earth billions of years ago when there was no oxygen in the atmosphere. To counteract the effects of UV radiation, cyanobacteria have developed various mechanisms, such as the production of UV-protecting compounds, antioxidants, and proteins. Cyanobacterial compounds have a wide range of applications in pharmaceutical and biotechnology firms. Plant extracts, pigments, proteins, and flavonoids are commonly used in the synthesis of nanoparticles (Khalifa *et al*., 2021).

Cyanobacteria have the ability to retain water and produce UV protective compounds, allowing them to be used as natural UV filters and moisturisers. They are also high in antioxidants, which can neutralise free radicals. Nanotechnology provides

amazing, remarkable, and innovative product delivery solutions. Solid lipid, liquid lipid, non-ionic surfactant , nanoemulsions, and microemulsions are new lipid carrier vehicles with high penetrating power, high stabilisation and entrapment efficiency, site-targeted drug delivery, and controlled and sustained drug release. Cyanobacterial-derived nanoparticles have produced better results due to their low cost, slow kinetics, and environmental friendliness (Derikvand *et al*., 2017).

Scytonemin, a yellow-brown pigment found in cyanobacterial extracellular sheaths, was discovered in species that thrive in high-light environments. Scytonemin was found primarily in the sheaths of the outermost parts of cyanobacterial mats, crusts, or colonies. Scytonemin appears to be a single compound found in more than 30 cyanobacterial species from cultures and natural populations. It is lipid soluble and has a strong absorption maximum in the near-UV region (384 nm in acetone), with a long tail extending into the IR region (R. W. *et al*., 1991).

The aphrodisiac activity of the hydroalcoholic extract of the fruits of *Cydonia oblonga* Miller in Wistar rats. Four groups of six rats each were formed from the sexually active male rats. A single dose of the extract was given orally every day for 28 days in doses of 500 mg/kg and 800 mg/kg body weight. Mounting frequency, mating performance evaluation, and orientation activities toward females, the environment, and self were the parameters observed. The results showed that administration of the extract significantly increased mounting frequency and rat mate performance (Aslam *et al*., 2014).

The performance of quince leaf decoction extract on testicular rupture and impaired sperm emission induced by hyperlipidemia in rabbits. Eleven mature New Zealand white bucks were randomly categorised into three classes: hypercholesterolemia (n =3), hypercholesterolemia plus quince treatment (n =6), and control (n =2). Group 2 received a *C. oblonga* leaf extract as a beverage for six weeks, while Groups 1 and 2 consumed a high-cholesterol diet. After six weeks, groups 1 and 2 switched to a regular diet for six weeks. Group 3 (the control group) continued to follow a regular diet for the duration of the study. After a light microscopic examination of the animal's resected testes at the end of the 12th week, they concluded that the quince leaf extract protected rabbit testes and spermatogenesis from damage caused by hyperlipidemia (Ashrafi *et al*., 2013).

The effects of *Cydonia oblonga* leaf ethanolic extract on hyperlipidemic renal injury in rabbits. Eleven adult New Zealand white buck rabbits were randomly divided into three groups and kept in constant laboratory conditions for two weeks prior to the study in terms of humidity, illumination, and temperature. A diet high in cholesterol was given to group 1. Group 3 received a regular diet without cholesterol enhancement or quince leaf decoction extract, while Group 2 received a cholesterol-enhanced diet along with *C. oblonga* leaf extract as a beverage. The research showed that cholesterol-fed rabbits suffered glomerular and tubular injuries, but the basement membrane remained intact. The glomerular and tubular injuries in cholesterol-fed animals treated with the quince leaf extract supplement were milder. Therefore, it is conceivable that *Cydonia oblonga* leaf protects the kidneys. It is concluded that the likely protective effects of quince leaf extract on hyperlipidemia-induced renal injury can be attributed to both antioxidant and lipid-lowering effects (Fazeenah *et al.*, 2016).

The anti-allergic activity of *Cydonia oblonga* fruit hot water extract in mice. The mice were divided into three groups and given diets with a control level of quince hot water addition, 2.5 percent quince hot water addition, and 5.0 percent quince HW addition, respectively. They looked at the growth of skin lesions resembling eczema in mice, immunoglobulin E levels in the serum, and the release of HEXB from a rat basophilic leukaemia (RBL) cell line. The study revealed that after three weeks, mice in the control group developed symptoms resembling eczema on their faces, ears, noses, necks, and dorsal skin, whereas the severity scores of the symptoms in *Cydonia*-treated mice were significantly lower. IgE levels in control and quince-treated animals were 1635 ±289 and 994 ±205 ng/ml, respectively, with a statistically significant difference ($P < 0.01$). They came to the conclusion that quince HW inhibited type I allergy by suppressing IgE production and IgE-medicated degranulation (Shinomiya *et al.*, 2009).

The immunomodulatory and anti-allergic properties of phenolic compounds in lemon and quince fruits in patients suffering from allergic disorders and compared them with azelastine and dexasone. The analysis indicated that the degranulation of basophilic cells was reduced only in the presence of lemon. Additionally, *Cydonia oblonga* and lemon inhibited the production of Interleukin-8 and Tumor Necrosis Factor α from human mast cells (Huber *et al.*, 2012).

A literature review on the potential for prophylactic and therapeutic effects of *Cydonia oblonga* leaves in reducing cardiovascular disease based on its benefical constituents, demonstrated in other investigations, including antihypertensive, antioxidant, anti-atherogenic, anti-inflammatory, and vasodilatory effects. The potential effectiveness of these compounds as preventative measures against cardiovascular disease includes their potential effects on antioxidant activity, inhibitory effect on xanthine oxidase (XO), and capacity to chelate metals; improvement of myocardial ischemic tolerance to reperfusion syndrome; reduction of low-density lipoprotein oxidation by antioxidant property and increase in high-density lipoprotein levels, primarily due to flavonoids; anti-atherogenic effects in vessels, increasing nitrogen monooxide (NO) bioavailability and reducing hypertension, endothelial dysfunction, and vascular hypertrophy (VH) through vasodilatory consequences; and decreasing cardiac mastocyte mediator release and cardiovascular inflammation.The study concluded that quince leaves are an excellent natural source of bioactive substances with primary antioxidative properties as well as other mechanisms of action (Vaez H *et al*., 2014).

The antioxidant activity of phenolic extracts of Chinese *Cydonia oblonga* Mill., quince, and apple fruits. The antioxidant roles of Chinese quince and quince phenolic extracts were superior to those of $C_{16}H_{18}0_9$(CGA), most likely due to their high levels of polymeric procyanidins and hydroxycinnamic derivatives (Hamauzu *et al*., 2005).

The antioxidant activity of *Cydonia oblonga* fruit and jam. They prepared methanolic extracts of *Cydonia oblonga* fruit and jam, separated them into phenolic and organic acid fractions, and then used high-performance liquid chromatography to analyse the results. Antioxidant activity was always higher in the phenolic fraction than in the complete methanolic extract. The antiradical activity of organic acid extracts was consistently the lowest, which appears to suggest that the phenolic fraction contributes more to the antioxidant potential of *Cydonia oblonga* fruit and jam (Silva *et al*., 2004).

Evaluation by comparing the antioxidant properties of methanolic extracts of *Cydonia oblonga* leaf to green tea. The antioxidant properties of quince leaf were studied for their ability to quench the stable free radical, the induced oxidative hemolysis of human RBC, and the phenolic content of *Cydonia oblonga* leaf was determined by High Performance Liquid Chromatography or ultraviolet methods. *Cydonia oblonga* leaf had a considerably higher reducing power than green tea (average scores of 227.8 ± 34.9 and

112.5 ± 1.5 g/kg dry leaf, respectively). Quince leaf extracts showed comparable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical antioxidant potential (EC_{50} average score of 21.6 ± 3.5 lg/ml), but considerably lower than green tea extract (EC_{50} average score of 12.7 ± 0.1 lg/ml). The amount of quince leaf methanolic extract increased under the oxidative influence of 2,2-azobis (2-amidinoprpoane) dihydrochloride (AAPH) (Costa *et al*., 2009).

The effect of methanolic extract of *Cydonia oblonga* leaf on buck in contrast to atovastatin. Twenty-four dollars were randomly divided into two groups: normal diet (n = 6) and high cholesterol diet (n = 18). Eight weeks later, all of the normal rabbits and three of the high-cholesterol rabbits were killed, and atherosclerosis was observed. The rabbits were divided into three groups: control, atovastatin 0.5 mg/kg, and *Cydonia oblonga* leaf extract 50 mg/kg. Blood samples from all three groups were collected at the end of three months, and biochemical parameters were determined.The outcomes demonstrated that there is no significant difference between the atovastatin and *Cydonia oblonga* extract groups, indicating that the cholesterol lowering drugs of *Cydonia oblonga* extract are the same as those of atovastatin (Khademi *et al*., 2013).

The effects of *Cydonia oblonga* juice and *Cydonia oblonga* hydroalcoholic extract on trinitrobenzenesulfonic acid (C6H3N3O9S) induced inflammatory bowel disease in rats. Six rats were grouped together and fasted for 36 hours before inflammatory bowel disease was caused. Trinitrobenzenesulfonic acid was embedded into the large intestine via a hydroalcoholic carrier, followed by 5 days of treatment with various doses of *Cydonia oblonga* juice (200, 400, and 800 mg/kg), *Cydonia oblonga* hydroalcoholic extract (200, 500, and 800 mg/kg) orally, *Cydonia oblonga* juice (400 mg/kg), and *Cydonia oblonga* hydroalcoholic extract (200 and 500 mg/kg) intraperitoneally. *Cydonia oblonga* juice and *Cydonia oblonga* hydroalcoholic extract were found to be effective in lowering ulcer index values (UI) and inflammation in this murine model of acute ulcerative colitis (UC) (Manaiyan *et al*., 2012).

The antibacterial activity of an ethanolic extract of quince seeds against *S. aureus, S. epidermidis, and E. coli*. *Cydonia oblonga* seeds have greater antibacterial activity against gram-positive bacteria than against gram-negative bacteria (Salehi *et al*., 2016).

The anti-helicobacter pylori effect and composition of various food plant extracts and juices in vitro in order to develop new functional food products with anti-H. afctivity.

Cydonia oblonga juice and cranberry juice were the two substances that showed the strongest anti-helicobacter pylori effect out of 33 substances, juices, and plant extracts that were tested, along with 35 combinations of these substances. The outcomes open up new avenues for the development of functional anti-H food products for the foodservice management of helicobacter pylori infection (Al-Snafi *et al*., 2016).

The antihypertensive effect of an ethanolic extract of fruit and leaves on renovascular hypertensive rats compared to that of captopril after eight weeks. Captopril had little effect on hemorheology, but *Cydonia oblonga* Milller extracts reduced whole blood viscosity and improved erythrocyte deformability (Zhou *et al*., 2014).

The antihypertensive effect of *Cydonia oblonga* Miller on renal hypertensive rats. For eight weeks, 60 hypertensive rats were randomly assigned to one of six groups: model, captopril control, *Cydonia oblonga* mill high, medium, and low three-doses, and sham operation. Rat's blood pressure was checked every 2 weeks. They discovered that the COM and Captopril groups were significantly lower than the model group ($P< 0.05$), and that theangiotensin-II levels in *Cydonia oblonga* Mill high, medium, and low dose groups were lower than the model group ($P< 0.05$) (Abliz *et al*., 2012).

The lipid-lowering and antihepatotoxicity effects of *Cydonia oblonga* Miller leaf extract on serum lipids and liver function in a rat model. 70 healthy rats were randomly divided into six groups: normal controls, model controls, simvastatin, and low, medium, and high dose COM leaf extracts, which were administered orally for fifty-six days. All other groups were fed a high-fat diet, while the normal controls were fed a normal diet. The weights of the rats were measured over a period of time. After 56 days, the serum lipid panel, as well as alanine transaminase, aspartate transaminase, and total protein, were measured. They discovered that *Cydonia oblonga* Miller had a similar effect to simvastatin, with the exception of LPL and hepatic triglyceride lipase, which were reduced by *Cydonia oblonga* Miller but not by simvastatin. Quince leaf extracts have lipid-lowering and antihepatotoxicity effects, which are likely due to increased antioxidant capacity and lipoprotein metabolic activity in the liver, as well as lipogenesis inhibition (Al-Snafi *et al*., 2022).

The anti-gastroesophageal reflux (GPS) effect of quince fruit extract. They conducted a pilot study on five infants who had reflux and did not respond to regular assessments. After a month, they discovered that 4 of the 5 infants had significant

changes in reflux symptoms such as agitation, vomiting, coughing, and poor appetite. They experienced soft defaecation with the extract and concluded that *Cydonia oblonga* Mill. is a good remedy for infant reflux, most likely due to its adstringent property, which can strengthen the urethral sphincter and stop reflux (Tansaz *et al*., 2013).

Natural substances are used to produce nanoformulations, which serve as an effective delivery system for the skin. These new generation nanoformulations improved stability, drug delivery, and interaction, and lipid nanoparticles demonstrated controlled drug release. Natural nanomaterial loaded topical formulations are suitable for application to the skin and provide a safer alternative with good efficacy for disease treatment. Some of the injectable nanoparticles that have hit the market are polymeric nanoparticles, associated colloids, endosperm, and a core-shell nanoparticle that avoids excessive drug circulation. Nanoformulations loaded with drugs should carry a large number of drugs for Ultravoilet B-induced skin cancer because of their ability to entrap lipophilic as well as hydrophilic drugs, respectively. Drug distribution NE ,ME, solid lipid nanoparticles, nanostructured lipid carriers, liposomes, and nonionic surfactant vesicles have the advantages of controlled and sustained drug release, site-specific targeting, high stability, and penetration power. Nanoparticle properties include size, shape, covering, toxic effects, and pureness. They influence skin permeation yet are effectively transported to blood vessels via intercellular and shunt routes. Liposomes can be applied topically or ingested orally. The efficacy of nanostructured lipid carriers is dependent upon temperature, percentage of oil incorporated into the lipid layer, and emulsifier composition, while liposomes achieve optimal therapeutic efficacy through continuous release of bioactive components (Tamjidi *et al*., 2013).

Oral nanoemulsions have previously been explored as potential vehicles for improving the therapeutic activities and bioavailability of a variety of bioactive molecules. Because of their thermodynamic stability and nanosized droplet range, nanoemulsions have several potential advantages over unstable dispersions. The effects of nanoemulsion in enhancing carbon monoxide antibacterial effects against clinical isolates of various bacterial species (Kale *et al*., 2017). They discovered that nanoemulsion drastically enhanced the antibacterial effects of carbon monoxide when compared to pure carbon monoxide. Numerous beneficial oils derived from various plant sources have also been studied for potential healing effects in various animal models. The excisional

wound healing rat model is the most widely used animal model to evaluate essential oil wound healing effects (Alam *et al*., 2018).

Nanoemulsions are emulsions with lipids that are primarily produced by high-energy or low-energy emulsification. NEs are also known as ultrafine emulsions because of the formation of droplets in the submicron range. The average droplet size of nanoemulsions has been defined as being between 50 and 500 nm, with a typical value of 200–300 nm. This is less than the typical 1-100 mm droplet size range in macroemulsions. Because of their small size, droplets can sustain physical destruction caused by gravitational separation, flocculation, or coalescence. Nanometer-size/diameter and polydispersity index influence emulsion properties such as particle stability, rheology, image, colour, texture, and shelf life, as well as improving drug pharmacological effects (Patel *et al*., 2012).

Hemostasis, inflammation, proliferation, and maturation are the four stages of normal wound healing. Macrophages are the most important inflammatory cells in wound healing because they phagocytose pathogenic organisms, degrade debris, and stimulate the formation of granulation tissue. Fibroblasts are required for proliferation and the formation of structural elements such as collagen, elastin, and extracellular matrix proteins. Type III collagen is converted to type I collagen during the remodelling process, which can take weeks to years. Mature scar strength is approximately 80% that of unwounded skin. Wound healing time is affected by a variety of factors, including wound size, depth, location, patient age, and local and systemic disease. Acute wounds heal normally and quickly through the stages of healing. Chronic wounds fail to heal in a normal, orderly, and timely manner, or without restoring normal anatomy and function. Cytokines, growth factors, and proteases all play a role in normal healing. Chronic inflammation, cellular senescence, a poor cytokine milieu, and critical bacterial colonisation all contribute to poor wound healing. Chronic wounds produce more cytokines and proteases, which destroy extracellular matrix components, growth factors, and growth factor receptors (Mast *et al*., 1992).

According to wound healing research, fibroblasts in acute, healthy wounds are more active and respond better to inflammatory responses than those in disordered wounds, either because the fibroblasts themselves are damaged as evidenced by a lack of response to growth factors or because the environment is unfavourable. In fibroblast

cultures, secretions from healthy wounds stimulate DNA synthesis, whereas those from chronic, non-healing wounds (e.g., leg ulcers) inhibit it. Indeed, fibroblasts thrived when the inhibitory effect of chronic wound fluid was removed by heating to denature the contents (Patel *et al*., 2016).

OBJECTIVES

1. Maintenance and culturing of *Scytonema javanicum* and extraction of scytonemin.
2. Preparation of *Cydonia oblonga* Mill. seed extract.
3. Development and characterization of the nanoemulsion loaded with the extracts.
4. Study of wound healing properties of *Cydonia oblonga* (CO) and scytonemin (SCY) loaded nanoformulation.

3. MATERIAL AND METHODS

3.1 Culturing of *Scytonema javanicum* and extraction of scytonemin

The axenic cultures of *scytonema javanicum* will be obtained from Algal Research Laboratory, University of Allahabad. Cells were allowed to grow in the BG-11 medium (Rippka *et al*., 1979) and buffered to pH 7.5 After every 28 days, sub-culturing was done by transferring test algae to fresh media under the same condition to maintain them in the exponential phase. Stock cultures were maintained on solid agar slants. The cultures were raised in bulk after those cells were harvested, and centrifuged; the harvested cells were washed with nitrogen-free medium and re-suspended in the same fresh medium. This was used as inoculum for all the experiments. The components of BG-11 medium are as follows:

Table 1: Media composition of BG-11 medium

Macroelements	**gm/l**
$MgSO_4.7H_2O$	**0.075**
$CaCl_2.2H_2O$	**0.036**
Na_2CO_3	**0.020**
EDTA (pH 8)	**0.001**
Citric Acid	**0.006**
Ferric Citrate	**0.006**
K_2HPO_4	**0.004**

Microelements	**Mg/l**
H_2BO_4	**2.860**

$MnCl_2.H_2O$	**1.810**
$NaMO_4.2H_2O$	**0.391**
$ZnSO_4.7H_2O$	**0.222**
$CuSO_4.5H_2O$	**0.079**
$Co(NO_3)2.6H_2O$	**0.049**

3.2 Extraction of scytonemin pigment from *Scytonema javanicum*

Fresh biomass from cyanobacterial strains was centrifuged and ground with a mortar and pestle to extract scytonemin. The cells were suspended in 100% acetone and incubated at 4 °C overnight. After centrifugation, the sample was filtered through Whatman filter paper 1, and the absorbance of the filtered contents was measured on a UV spectroscope at 720nm (Mushir S *et al*., 2014).

The following equation was used to calculate the scytonemin content:

$$A*384\ (Scyt) = 1.04A384 - 0.79A663 – 0.27A490$$

A is the absorbance measured at A.

In the absence of a scytonemin absorption coefficient, the unit represents the absorbance of 1mg fresh weight of material extracted in 1 ml acetone in a cuvette with a path length of 1 cm. Specific content is denoted by (Aλ. μg-1).

3.3 Collection of *Cydonia oblonga* Mill. Seeds

Seeds of *C. oblonga* were purchased from a local grocery store in Lucknow, India.

3.4 Extraction of *Cydonia oblonga* Mill.

The seeds were finely ground for extraction. Methanol was used as the solvent for the extraction of *Cydonia oblonga* Mill. The Soxhlet apparatus was used to isolate Quince seed extract for 24 hours at temperatures below the solvent's boiling point. After filtering,

the solvent was evaporated at room temperature and the residue was used for further analysis. The final extract was stored for in-vitro analysis.

3.5 Preparation of Nanoemulsion from both the extracts

Solubility studies

The most important component screening criterion is the solubility of poorly soluble drugs in oils, surfactants, and cosurfactants (Akhter et al., 2008). The solubility of scytonemin-*Cydonia oblonga* extract in olive oil, Sefsol 218, and oleic acid was investigated. 2 mL of various oils were placed in tiny vials along with an excess of medication. After being agitated for 72 hours at 37 0.5 ˚C, the vials were tightly sealed and centrifuged for 10 minutes at 10,000 rpm.

The solubility was measured using UV spectroscopy after filtering the suspension with a 0.45 m membrane filter and diluting it with ethanol. To optimise surfactant and cosurfactant, various rations such as 1:0, 1:1, 1:2, and 2:1 were used, and the mixture was dubbed Smix. For each phase diagram, oil and specific Smix were thoroughly mixed in various percentages. Four different Smix ratios (1:0, 1:1, 1:2, and 2:1) were used to create phase diagrams. The phase diagram was created using aqueous titration.

3.5.1 Construction of Pseudo Ternary Phase Diagram

Pseudo-ternary phase diagrams of oil, water, and cosurfactant/surfactant mixtures were created at fixed cosurfactant/surfactant weight ratios. The materials were mixed, pre-weighed into glass vials, titrated with water, and thoroughly stirred at room temperature to create the phase diagrams. The formation of the monophasic/biphasic system is confirmed visually. If the samples exhibit turbidity followed by phase separation, they are classified as biphasic. After stirring, monophasic, clear and transparent mixes should be examined; the samples should be marked as points on the phase diagram.

The aqueous titration method was used to generate pseudo ternary phase diagrams from oil, Smix (surfactant-co-surfactant combination), and double distilled water. Surfactant and cosurfactant were mixed in different volume ratios (1:0, 1:1, 1:2, and 2:1), as cosurfactant concentration increased relative to surfactant and vice versa. Each phase diagram has its own oil and phase diagram.

Thirteen different oil and Smix combinations were titrated with aqueous phase and evaluated visually for transparency and flowability (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:9, 2:1, 3:1, 4:1, 5:1,6:1, 7:1). The phase diagrams were used to depict the nanoemulsion's physical state, with three phases denoting an aqueous phase, oil, and Smix. For each phase diagram, the nanoemulsion area was plotted, and the larger the region, the better the results were.

3.5.2 Methods of Preparation of Nanoemulsion

A series of Nanoemulsion (NE) formulations were developed using Sefsol 218 as an oil, Tween 80 as a surfactant, and ethanol as a cosurfactant. For each phase diagram, Smix ratios (1:0, 1:1, 1:2, and 2:1) were calculated. Various ratios of oil and particular Smix were blended well. Twenty-three different combinations of water, oil, and Smix were visually examined for phase diagram construction. A distinct phase diagram was created for each Smix ratio.

3.5.3 Aqueous Titration Method

The drug was mixed with a binary mixture of surfactant and co-surfactant in a titration tube. The titration table was used to adjust the oil-to-binary-mixture ratio.For a few minutes, the titration tube was placed in a vortex and various concentrations were evaluated.The concentration at which a bluish hue appears was labelled as the concentration at which nanoemulsion production occurred at that particular oil and symmetric combination concentration. Several formulations were tested for stability and dispersibility.

3.6 Measurement of Particle Size

Average particle size (Z-average) of the developed nanoparticles were determined by laser Dynamic Light Scattering using Malvern Zetasizer (Nano ZS, Malvern Instruments, UK).

3.6.1 Thermodynamic Stability Studies

Nanoemulsions are thermodynamically stable systems that form at a specific oil concentration, surfactant-co-surfactant ratio, and water concentration, with no phase

separation, creaming, or cracking. Thermodynamic stability confers a long shelf life to the nanoemulsion as compared to ordinary emulsions. It differentiates them from emulsions that have kinetic stability and will eventually phase-separate. Selected formulations were subjected to different thermodynamic stability tests to assess their physical stability. The following tests were used to determine the thermodynamic stability of drug-loaded nano-emulsions:

- **Heating–cooling cycle:** Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48 h were conducted, and the formulations were examined for stability at these temperatures.
- **Centrifugation test:** Formulations were centrifuged at 5000 rpm for 30 min, and we looked for phase separation.
- **Freeze–thaw cycle:** Three freeze–thaw cycles between −20°C and +20°C, with formulation storage at each temperature for not less than 24 h, were performed.

3.6.2 Fourier-transform infrared spectroscopy (FTIR) analysis

Infrared (IR) transmission measurements were taken between 4000 and 650 cm^{-1} with an FTIR/FIR spectrophotometer version 10 (Perkin Elmer, Waltham, MA, USA) and a KBr beam splitter. The Scytonemin-*Cydonia oblonga* extract was fused with oven-dried potassium bromide (stored in a desiccator) in a 1:100 ratio. The samples were presented in the form of a transparent KBr disk, and the spectra were recorded at a resolution of 4 cm^{-1} with 64 scans.

Fourier transform infrared spectroscopy (FTIR) helped to verify the entrapment of the drug and to check the interaction of the drug with lipids in formulation. An FT-IR spectrophotometer (8400S, Shimadzu, Japan) equipped with an attenuated no of formulation and pure extract.FT-IR spectral measurements for pure Scytonemin-*Cydonia oblonga* extract and nanoformulations, respectively, were taken at ambient temperature. All the samples were dried under vacuum prior to obtaining the spectra, in order to remove the effect of residual moisture (Shete and Patravale, 2013) for each spectrum, a resolution of 4 cm^{-1} from a frequency range of 4000–600 cm^{-1} was taken.

3.6.3 Transmission electron microscopy (TEM) analysis

The morphology of the Scytonemin-*Cydonia oblonga* and Scytonemin-*Cydonia oblonga* loaded nanoformulation was observed using a transmission electron

microscope (model TECNAIG2, Philips, Eindhoven, The Netherlands). The extract and nanoformulation were then diluted 1:5 times and placed on copper grids with 2% (w/v) phosphotungstic acid staining for 40 seconds before further analysis.

3.7 Cell culture:

The HaCaT cell line was grown in Dulbecco's Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12 Ham) culture medium supplemented with 10% Fetal bovine serum (FBS), antibiotics, and antimycotic solution 1.5% at 5% CO2 and 95% relative humidity at 37˚C.

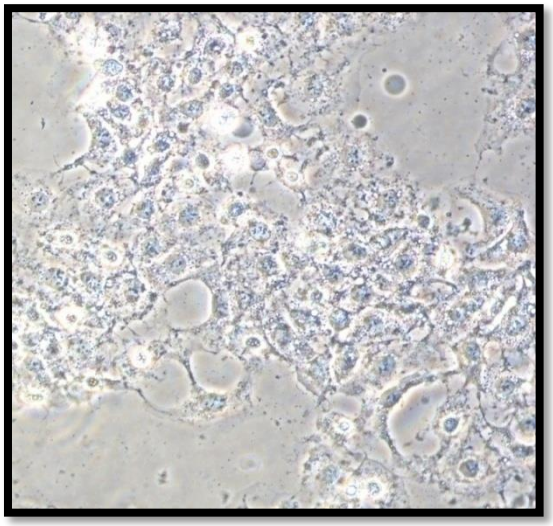


Fig. 1: The human skin keratinocyte cell line (HaCaT) as seen at 20X.

3.7.1 Chemicals used:

Sigma Chemical Company (St. Louis) provided Foetal Bovine Serum (FBS), Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12), antibiotic and antimycotic solution, Trypsin EDTA solution (Ethylenediamne tetraacetic acid) 1X, MTT (3-(4, 5-dimethylthiozolyl-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide) and DCFH-DA (2'-7'-dihydrofluorescein diacetate. The study used Milli-Q double-distilled deionized water.

3.7.2 The effect of Scytonemin-*Cydonia oblonga* extracts and their nanoemulsion on cell viability

Human adult keratinocytes (HaCaT cells) were cultured in DMEM F-12 supplemented with 12% FBS and 1% penicillin-streptomycin. The cells were maintained in a humidified incubator 5% CO2 at 37ºC. On reaching confluence, the cells were harvested and plated for subsequent passages. HaCaT cell suspensions (1 x 10^4 cells/ mL) were seeded into 96-well plates at a density of 8000 cells per well and cultured for 24 h as described previously. The culture medium was removed and replaced with fresh DMEM (without FBS) containing nanoemulsion formulations, extract and to obtain Scytonemin – *Cydonia oblonga* concentrations in sets of 3 wells of 5, 10, 20, 30, 50, 60, 70, 80, 90 and 100µg/ml. The cells were further incubated with 24h and 48 h. The viability of cells following exposure to Scytonemin-*Cydonia oblong*a extract -loaded nanoemulsions, Scytonemin-*Cydonia oblong*a extract (100 µg/ml in DMSO), placebo control (cells treated with nanoemulsion without Scytonemin-*Cydonia oblonga* extract) was determined using the MTT assay and compared with untreated controls (untreated cells).The assay functions on the basis that metabolically active cells are able to convert the yellow MTT dye [3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] into water-insoluble dark blue formazan crystals by reductive cleavage of the tetrazolium ring. Ten microliters of MTT dye solution (5 mg/mL in phosphate buffered saline) was added to each well, and the plates were incubated at 37º C for 3 h in a humidified 5% CO2 atmosphere. Subsequently, the medium was removed and the formazancrystals were solubilized with 100 mL of DMSO. The absorbance of each well was read at 570 nm using a microplatereader (iMark™ Microplate Absorbance Reader, Biorad), and cell viability was calculated using the following equation:

% Cell Viability= (Mean absorbance in test well/Mean absorbance in untreated control cells) x100

3.7.3 *In- vitro* wound healing assay

Briefly, HaCaT cells (5×10^4) were seeded into six-well cell culture plates and allowed to grow to 70–80% confluence as a monolayer. The monolayer was gently scratched across the center of the well with a sterile one-mL pipette tip. After scratching, the medium was removed, and the wells were washed twice in PBS (Sigma-Aldrich, Milan, Italy) solution. Fresh medium containing 5% V/V of heat-inactivated FBS were added and cells treated

with Scytonemin-*Cydonia oblonga* extract and its encapsulated nanoemulsion was added to each well, and cells were grown for 24 hours and 48 hrs. Images were obtained from the same fields immediately after scratching (t_0) and after 24 hours using Olympus microscope and images was analyzed using ImageJ software by manually selecting the wound region and recording the total area.

The experiments were conducted in triplicate, and two fields were analyzed for each replicate ($n = 6$). Untreated scratched cells represented the control.

The percentage of wound closure was calculated using the following formula:

$[(\text{Wound area } t_0 - \text{Wound area t})/\text{Wound area } t_0] \times 100$

3.7.4 Intracellular ROS generation by Microscopy:

ROS generation was assessed in HaCaT cells using 2', 7'-diclorodihydrofluorescein di-acetate (DCFH-DA, Sigma Aldrich, Missouri, USA) dye as fluorescence agent. Briefly, cells ($1x10^6$) were seeded in 6 well plates and treated with Scytonemin-*Cydonia oblonga* extract and Scytonemin-*Cydonia oblonga* extract loaded nanoemulsion at a concentration 92and 98 µg/ml for 24 and 48 hr. Cells incubate with 30 $mgmL^{-1}$ of DCFHDA dye for 30 minutes in dark at room temperature.The cells were then washed with PBS buffer. The production of ROS can be measured by changes in fluorescence due to the intracellular accumulation of DCF caused by the oxidation of DCFH. Intracellular ROS, as indicated by DCF fluorescence, was measured with a fluorescence microscope (Hseu *et al.,* 2012).

3.7.5 Cell Migration Assay:

In Boyden chamber cell migration assay, the Transwell (Millipore, Inc.) was coated with 0.1% gelatin for 30 min in cell incubator. Confluent HaCaT cells were detached by trypsin-EDTA, washed once, and re-suspended in DMEM medium containing 0.5% FBS. Cell suspensions (100 µl, 40,000 cells) were added to the upper chambers (the inserts) of the Boyden chamber system. The bottom chambers of Transwell were filled with DMEM media with 1% FBS. 8 to 10 h after migration, cells on the top surface of the membrane (non-migrated cells) were scraped with a cotton swab and cells spreading on the bottom side of the membrane (invasive cells) were fixed with cold 4% paraformaldehyde for 30 min. Migrated cells were stained with crystal violet. Images were taken using Olympus inverted microscope and invasive cells were quantified by manual counting.

4. RESULTS AND DISCUSSION

4.1. Maintenance of cyanobacterial strain, *Scytonema javanicum* and evaluation of scytonemin pigment

Cultures were maintained at 25°C±1°C under a white fluorescent tube of intensity 30-40 μEm -2 s -1 with a 14:10 light and dark cycle. Scytonemin is the main sunscreen pigment which plays an important role in UV protection. The heterocystous strains *Scytonema javanicum* used for the evaluation of scytonemin pigment. In *S. javanicum* (720μg/gm fresh weight) were found.

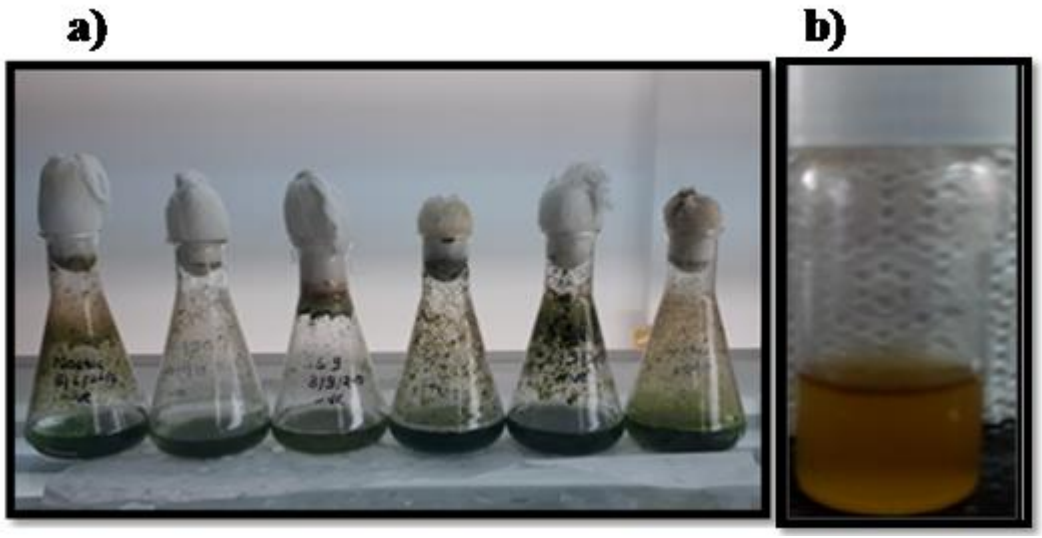


Fig. 2: a) Maintenance of cyanobacterial cultures b) Scytonemin extract.

4.2 Preparation of *Cydonia oblonga* Mill. extract

For the extraction, the seeds were finely powdered. The seeds were ground to fine powder in grinder and was extracted with 100 percent methanol. Quince seed extract was refluxed for 24 hours at temperatures below the boiling point of the solvent using the Soxhlet apparatus.

Scytonemin was extracted with 100% acetone using the Whatman filter No.1. The solvent was evaporated at room temperature after filtration, and the residue was used for further research (Fig 1). The Scytonemin-*Cydonia oblonga* extract was prepared at an appropriate ratio after filtration. The final Scytonemin-*Cydonia oblonga* extract was saved for further analysis.

Fig. 3: Preparation of *Cydonia oblonga* Mill. extract using Soxhlet apparatus.

4.3 Preparation of NE

Based on the screening of Smix ratios with a pseudo phase diagram, the ratio 2:1 (Table 1) produced the best nanoemulsion among all those made with different surfactant compositions, concentrations, co-surfactants, and water percentages with drug (Fig. 4(a) and Fig. 4(b)).

Fig. 4(a): Quince extract, Scytonemin extract and SCY-CO nanoemulsion (NE).

Fig: 1(a) Soxhlet extraction

Fig: 2(a) *Scytonema javanicum* culture

Fig: 1(b) Quince extract

Fig: 2(b) Scytonemin extract

Fig. 3: NE preparation (1:1)

Fig. 4(b): Preparation of SCY-CO extracts-loaded nanoemulsion.

Table 2: Composition of Selected Nanoemulsions

Smix(2:1)	Smix(%)	Oil (%)	Water (%)	Extract (%)
1:5	4.13	0.83	95.04	7.5

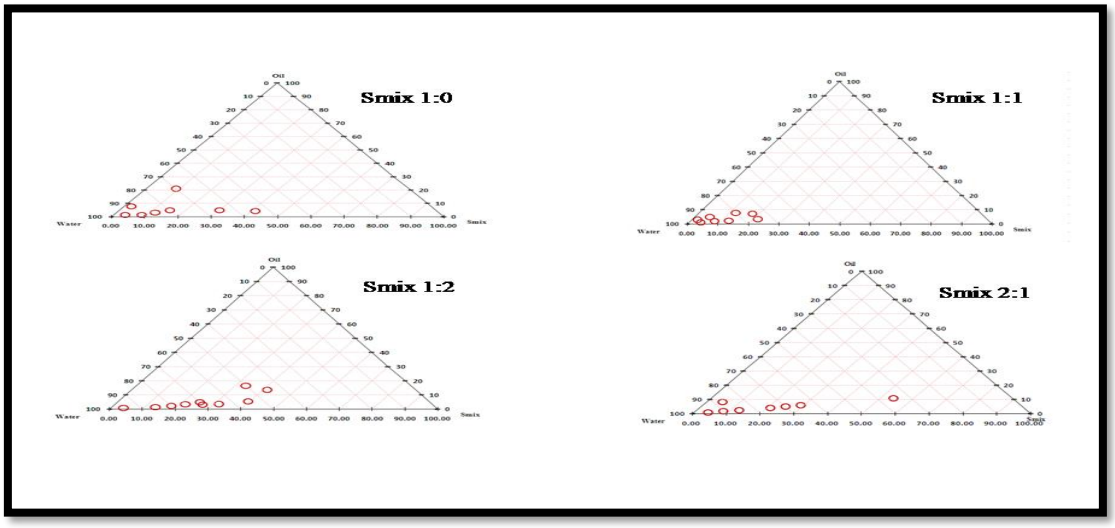


Fig. 5: Pseudoternary phase diagrams of Smix ratios; Smix 1:0, Smix 1:1, Smix 1:2, Smix 2:1.

4.4 Thermostability Stability Tests

Table 3: Thermodynamic Stability Tests

Formulations	S_{mix} ratio	%Oil	%S_{mix}	%Water	Thermostability studies tests			Dispersibility tests		Results
					Heating/ Cooling	Centrifugation	Heat/ thaw	0.1 N	H_2O	
NE_1	1:0	1.67	3.33	95.00	X	-	-	B	B	Failed
NE_2		1.64	8.20	90.16	✓	✓	✓	A	A	Passed
NE_3		5.00	15.00	80.00	✓	✓	x	B	C	Failed
NE_4		8.00	2.00	90.00	✓	✓	✓	C	D	Failed
NE_5		4.55	40.91	54.55	X	-	-	C	C	Failed
NE_6		5.00	30.00	65.00	✓	✓	x	B	C	Failed
NE_7		20.90	8.96	70.15	✓	✓	-	C	B	Failed
NE_8		3.33	11.67	85.00	X	-	-	D	C	Failed
NE_9	1:1	2.86	1.90	95.24	✓	X	-	B	C	Failed
NE_{10}		5.00	5.00	90.00	X	-	-	D	A	Failed
NE_{11}		8.00	12.00	80.00	X	-	-	C	A	Failed
NE_{12}		7.50	17.50	75.00	✓	✓	✓	A	A	Passed
NE_{13}		1.25	3.75	95.00	✓	✓	-	C	C	Failed
NE_{14}		2.22	7.78	90.00	✓	-	-	C	C	Failed
NE_{15}		2.50	12.50	85.00	✓	✓	✓	D	C	Failed
NE_{16}		3.57	21.43	75.00	✓	X	-	B	D	Failed
NE_{17}	1:2	1.11	3.89	95.00	✓	X	-	B	C	Failed
NE_{18}		1.67	13.33	85.00	✓	✓	x	C	C	Failed
NE_{19}		2.50	17.50	80.00	✓	✓	✓	A	A	Passed
NE_{20}		3.57	21.43	75.00	✓	✓	x	A	C	Failed
NE_{21}		3.51	31.58	64.91	X	-	-	B	D	Failed

NE_{22}		5.62	39.33	55.06	✓	✓	✓	D	C	Failed
NE_{23}		16.67	33.33	50.00	✓	X	-	C	D	Failed
NE_{24}		3.33	26.67	70.00	X	-	-	C	B	Failed
NE_{25}		5.00	25.00	75.00	✓	X	-	D	B	Failed
NE_{26}		13.70	41.10	45.21	X	-	-	C	A	Failed
NE_{27}	2:1	3.00	8.00	90.00	✓	✓	✓	D	C	Failed
NE_{28}		0.95	3.81	95.24	✓	X	-	C	A	Failed
NE_{29}		2.50	12.50	85.00	✓	✓	✓	C	D	Failed
NE_{30}		20.90	8.96	70.15	X	-	-	A	B	Failed
NE_{31}		18.00	2.00	80.00	X	-	-	A	C	Failed
NE 32		20.00	5.00	75.00	✓	✓	x	B	C	Failed
NE_{33}		0.83	4.13	95.04	✓	✓	✓	A	A	Passed

4.5 Analysis of particle size

Different concentrations of emulsifiers and co-surfactants at different mass ratios were used to optimize the stable nanoemulsion. The conditions for the preparation of nanoemulsion were optimized and stable nanoemulsion was obtained by sonification for 15 min.

The mean particle size of Scytonemin-*Cydonia oblonga* loaded nanoemulsion was found to be 200 nm.

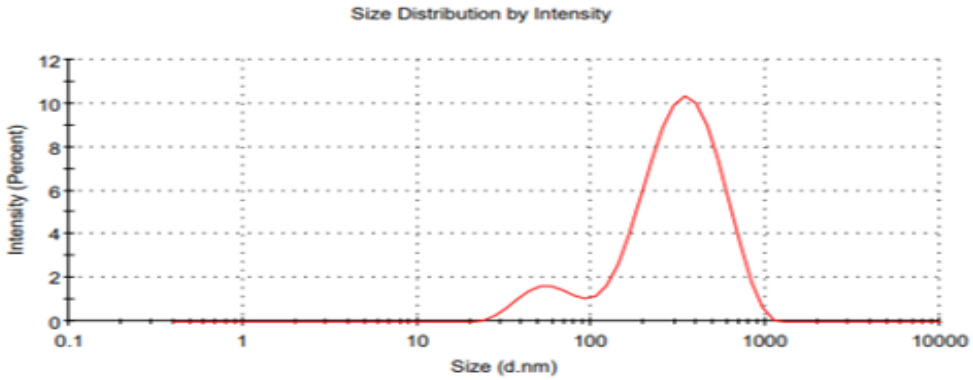


Fig. 6: Particle size SCY-CO nanoemulsion.

4.5.1 Entrapment of drug study by FTIR Spectroscopy

The FTIR spectrum of Scytonemin-*Cydonia oblonga* extract (Fig.7(a) and Fig.7(b) exhibited a broad absorption at 3418 cm^{-1} which corresponds to the phenol group. Absorbance at 2923 cm^{-1} is typical of C–H stretching vibrations of alkanes. C–O stretching was generally observed at 1083 cm^{-1}. Nanoformulations showed characteristic peaks of their excipients utilized, as shown in figure, but sharp peaks of scytonemin extract of *S. javanicum* was absent which may be due to complete entrapment of the extract inside the system as shown in Fig.7(a) and Fig. 7(b).

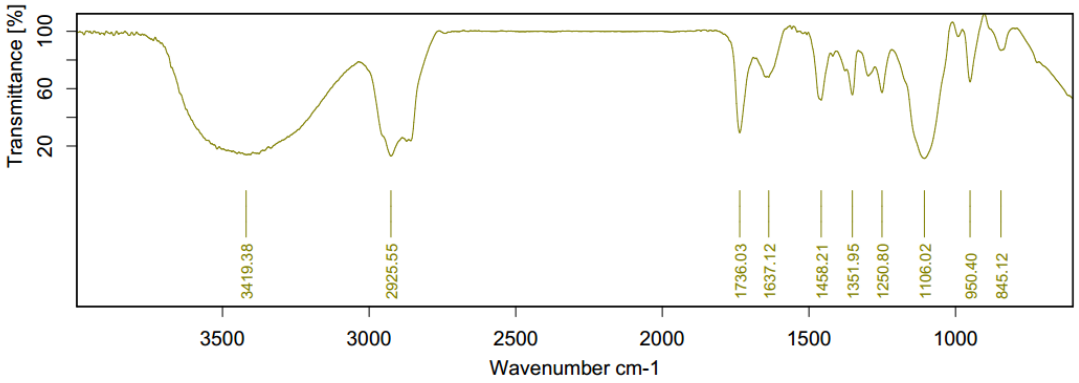


Fig. 7(a): FTIR spectrum of the SCY-CO extract.

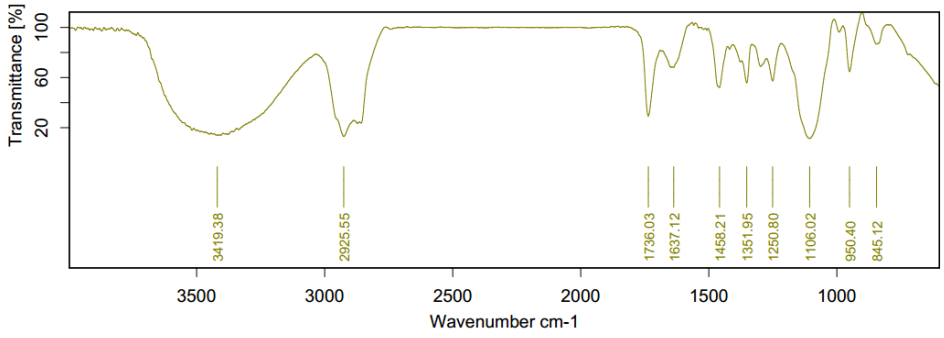


Fig. 7(b): FTIR spectrum of the Scytonemin-*Cydonia oblonga* NE.

4.5.2 Transmission electron microscopy (TEM)

In order to get some additional information on the morphology and particle size, TEM analysis was also performed on of Scytonemin-*Cydonia oblonga* extract loaded nanoemulsion (Fig. 9). As anticipated, particles found to be a spherical shape with varying size ranging approximately from 90 to 120 nm. Compared to diameter determined by DLS, the diameter observed by TEM was smaller, which might be explained by saying that the two methods were depending on different batches of samples and diverse principles. These discrepancies might be resulted due to lipid compositions and surfactants and/or due to the photographic tools used.

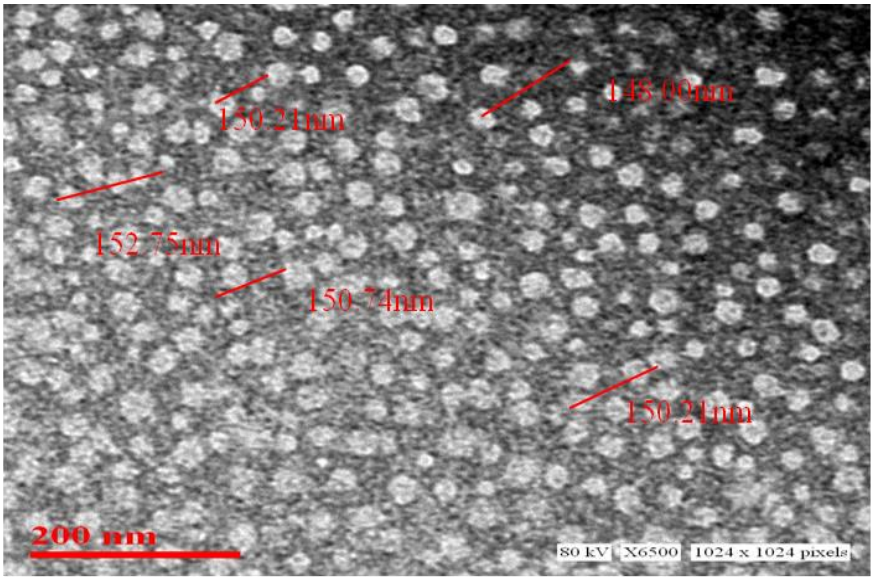


Fig 8: TEM micrographs of Scytonemin-*Cydonia oblonga* nanoemulsion.

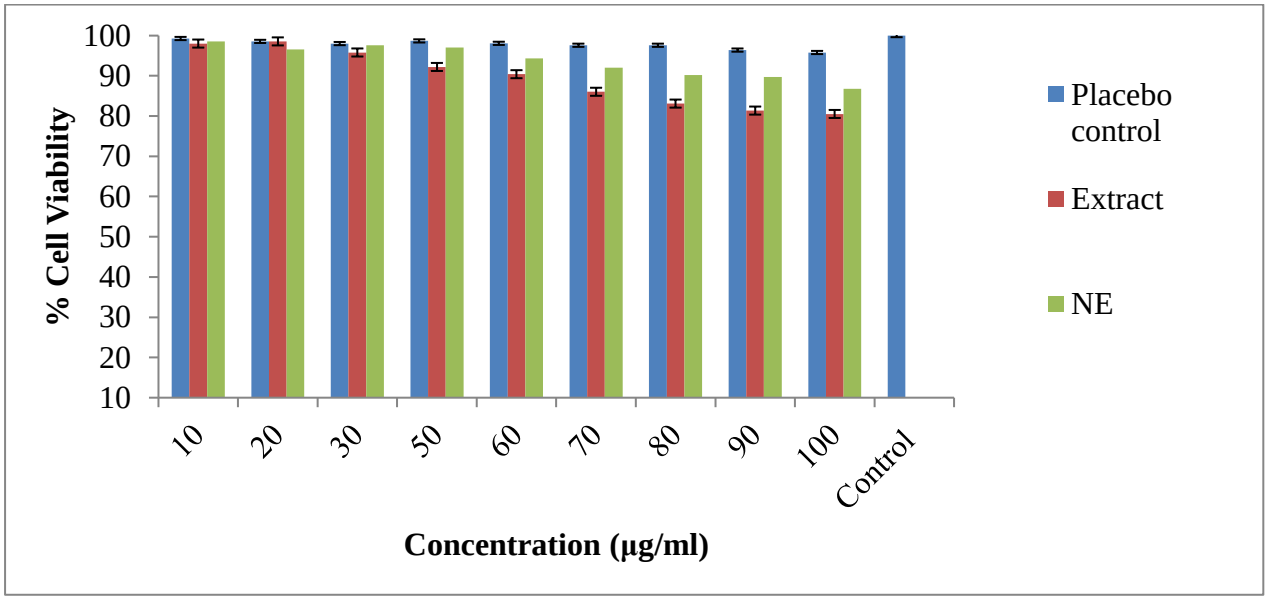
4.6 Effects of scytonemin-*Cydonia oblonga* extract and its nanoemulsion on human skin cell viability

The results obtained from the MTT assay on the HaCaT cells are given in Fig.9 and revealed that Scytonemin *Cydonia oblonga* extract and formulated nanoemulsion (at all the concentrations tested) exhibited negligible cytotoxicity towards the HaCaT cells. The scytonemin *Cydonia oblonga* extract and nanoemulsion showed IC_{50} value 92µg/ml and 98 µg/ml respectively at 24 hr while at 48hr the Scytonemin-*Cydonia oblonga* extract and formulated nanoemulsion showed IC_{50} value <100 µg/ml as given in Table 4. An IC_{50} value >100µg/ml is considered least toxic and least toxic to the cells. Here, IC_{50} value almost equals to the 100 µg/ml at 24 hr and at 48hr the extract and nanoemulsion showed

IC50 value greater that 100 µg/ml. So, the Scytonemin *Cydonia oblonga* extract and formulated nanoemulsion showed time dependent as well dose dependent effect. Both are also least cytotoxicity towards the HaCaT cells. It is also shown that Scytonemin *Cydonia oblonga* nanoemulsion showed least cytotoxicity as compared to the extract.

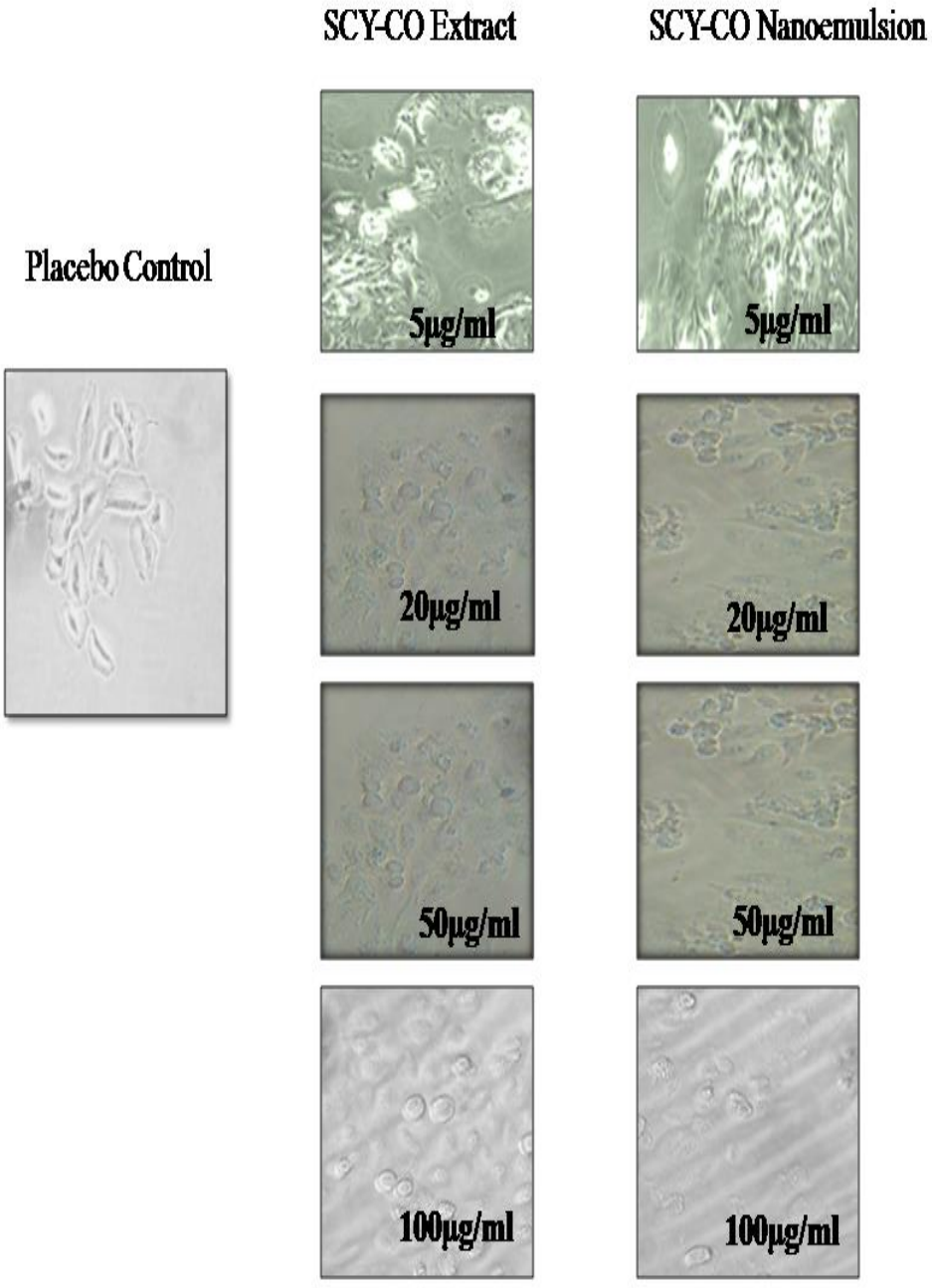
Table 4: %cell viability in terms of (IC50 ± SEM in µg/ml)

IC50 Value (µg/ml)		
Treatments	**24h**	**48hr**
Scytonemin-*Cydonia oblonga* extract	92	<100
Scytonemin-*Cydonia oblonga* extract loaded nanoemulsion	98	<100
Nano emulsion without extract	<100	<100



MTT-cell viability data shows the dose-dependent effect of Scytonemin-*Cydonia oblonga* extract and its nanoemulsion on the viability of human keratinocytes (HaCaT cells) after 24 h of exposure. The mean and standard deviation of three experiments were expressed as a percentage of cell viability when compared to untreated cells. Positive control refers to the cells treated with (10% FBS and DMEM F-12 medium). The viability of the untreated control was similar to that of the placebo control (cells treated with

nanoemulsion without extract). The results are significantly different from the untreated control. Nanoemulsions are significantly different from extracts at equivalent concentrations of scytonemin and *Cydonia oblonga*.



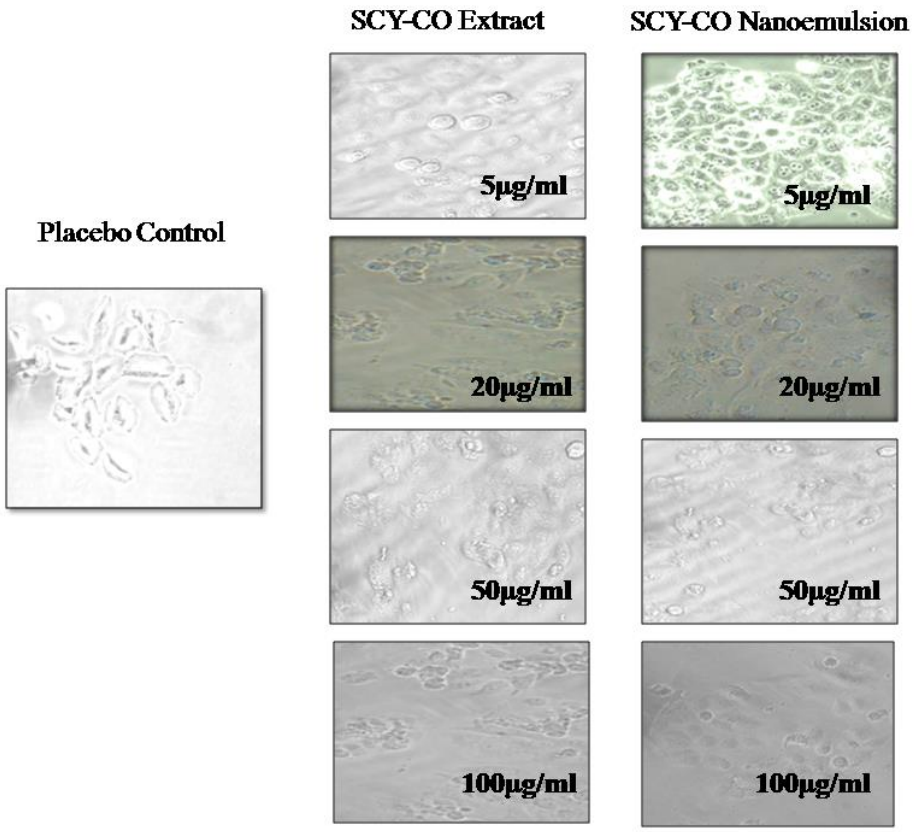


Fig. 9: Cell morphology analysis of scytonemin-*Cydonia oblonga* extract and scytonemin-*Cydonia oblonga* nanoemulsion for 24 hr and 48 hr, respectively.

4.6.1 Evaluation the effects of Scytonemin-*Cydonia oblonga* extract and its nanoemulsion on wound healing assay

Results of the scratch assays are given in Figs. 10(a) and 10(b). In general, when using Human Keratinocytes cells (HaCaT), NEs with incorporated active ingredients led to significantly smaller cell free gaps after 24h and 48 h in most cases (Fig.10). An example of an *in vitro* scratch assay after treatment with the loaded and unloaded NE containing sefsol oil as the oil phase.Surprisingly, NEs incorporated with the well-established wound healing drug scytonemin-*Cydonia oblonga* extract did not show superior wound healing properties when compared to NEs with scytonemin-*Cydonia oblonga* nanoemulsion as the active ingredient. NE loaded with scytonemin-*Cydonia oblonga* extract was able to close the artificial wound significantly better than the NE loaded with scytonemin-*Cydonia oblonga* extract. In the case of the NEs with sefsol oil, NE with an extract showed significantly smaller cell free gaps than the alone scytonemin-*Cydonia oblonga* extract. Scytonemin-*Cydonia oblonga* extract showed wound closure: 74 and 79 % at 24

and 48 h respectively while Scytonemin-*Cydonia oblonga*extract loded nanoemulsion showed wound closure 85 and 90 % respectively which was significantly higher than the wound closures obtained with the extract. It is found that some oils may significantly influence wound healing as vehicle compounds themselves. Additionally, the release of the extract from the vehicle oil can either enhance or impair wound healing and a complex triangular interaction between wound, vehicle are assumed. However, most wound healing experiments have been carried out with immortalised HaCaT cell lines. Due to substantial interaction and cooperation between the two cell types during the wound healing process, it is conceivable that the following reported responses from keratinocytes may be mirrored or supported in our scratch assays. This finding could explain in part the observed wound healing effects of Quince. It presumably stimulates fibroblasts of the tissue around the wound to proliferate, expresses appropriate integrin receptors and migrates into the wound space. In fact, the appearance of fibronectin and appropriate integrin receptors that bind fibronectin, fibrin, or both on fibroblasts appears to be the rate-limiting step in the formation of granulation tissue. Enhancement of fibroblasts proliferation indicates that Quince may contain growth promoting factor(s). These components are likely to candidates for the effects of quince on proliferation of fibroblasts in the present study. Quince seed presents a phenolic profile composed of 3-O-caffeoylquinic, 4-O-caffeoylquinic, 5-O-caffeoylquinic and 3, 5-dicaffeoylquinic acids, lucenin-2, vicenin-2, stellarin-2, isoschaftoside, schaftoside, 6-C-pentosyl-8-C-glucosyl chrysoeriol and 6-C-glucosyl-8-C-pentosyl chrysoeriol. The caffeoylquinic acid is a potent antioxidant. Radical scavenging and antioxidant activity of quince seed may explain the accelerative effect of quince on fibroblast proliferation. Skin provides a mechanical and immunological barrier between the body and the environment, and dysregulated inflammatory reactions in the skin can cause a variety of skin diseases . Exacerbated inflammation is a hallmark of a variety of inflammatory skin diseases, including dermatitis, psoriasis, and rosacea. Skin inflammation is also known as a key process involved in tumorigenesis and wound healing in the skin .Therefore, it is required to down-regulate exacerbated or persistent inflammation of the skin to prevent these pathological conditions. In this study, we investigated the anti-inflammatory effect of scytonemin in a mouse model of skin inflammation. Still, further research on the exact mode of action, especially in the later phases of the wound healing process, is of eminent interest and might provide important insight into the wound healing activity of Scytonemin-*Cydonia oblonga* nanoemulsion (Vater *et al*., 2022).

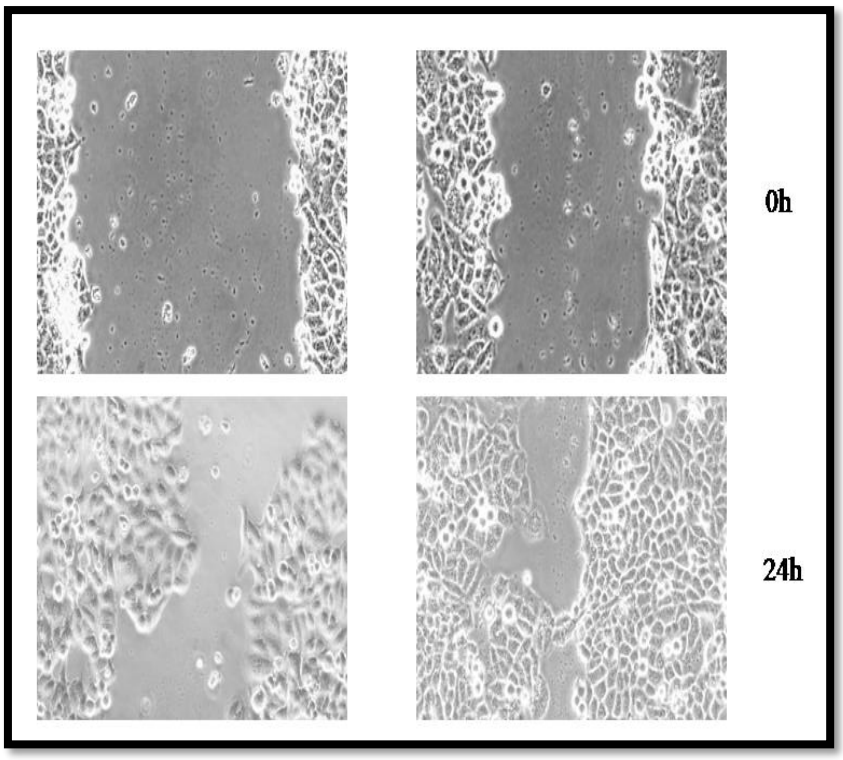


Fig. 10(a): In vitro scratch assay of HaCaT cells with unloaded and scytonemin-*Cydonia oblonga* extract and scytonemin-*Cydonia oblonga* nanoemulsion loaded containing jojoba oil as the oil phase for 0h and 24h.

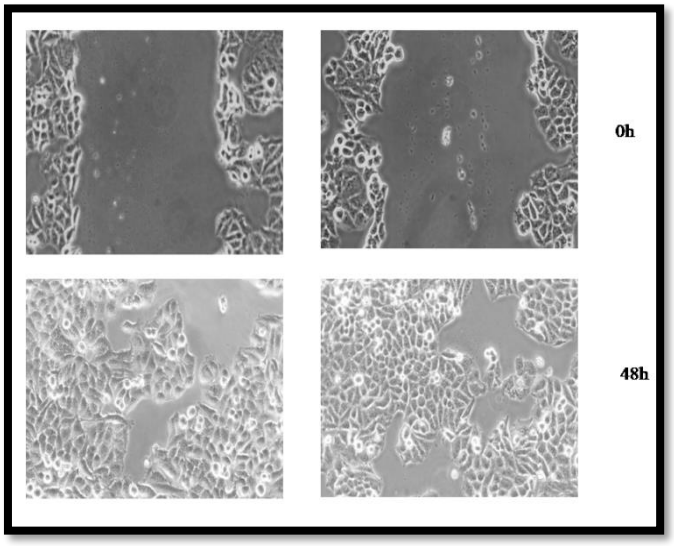


Fig. 10(b): *In vitro* scratch assay of HaCaT cells with unloaded and scytonemin-*Cydonia oblonga* extract and scytonemin-*Cydonia oblonga* nanoemulsion loaded containing sefsol oil as the oil phase for 0h and 48h.

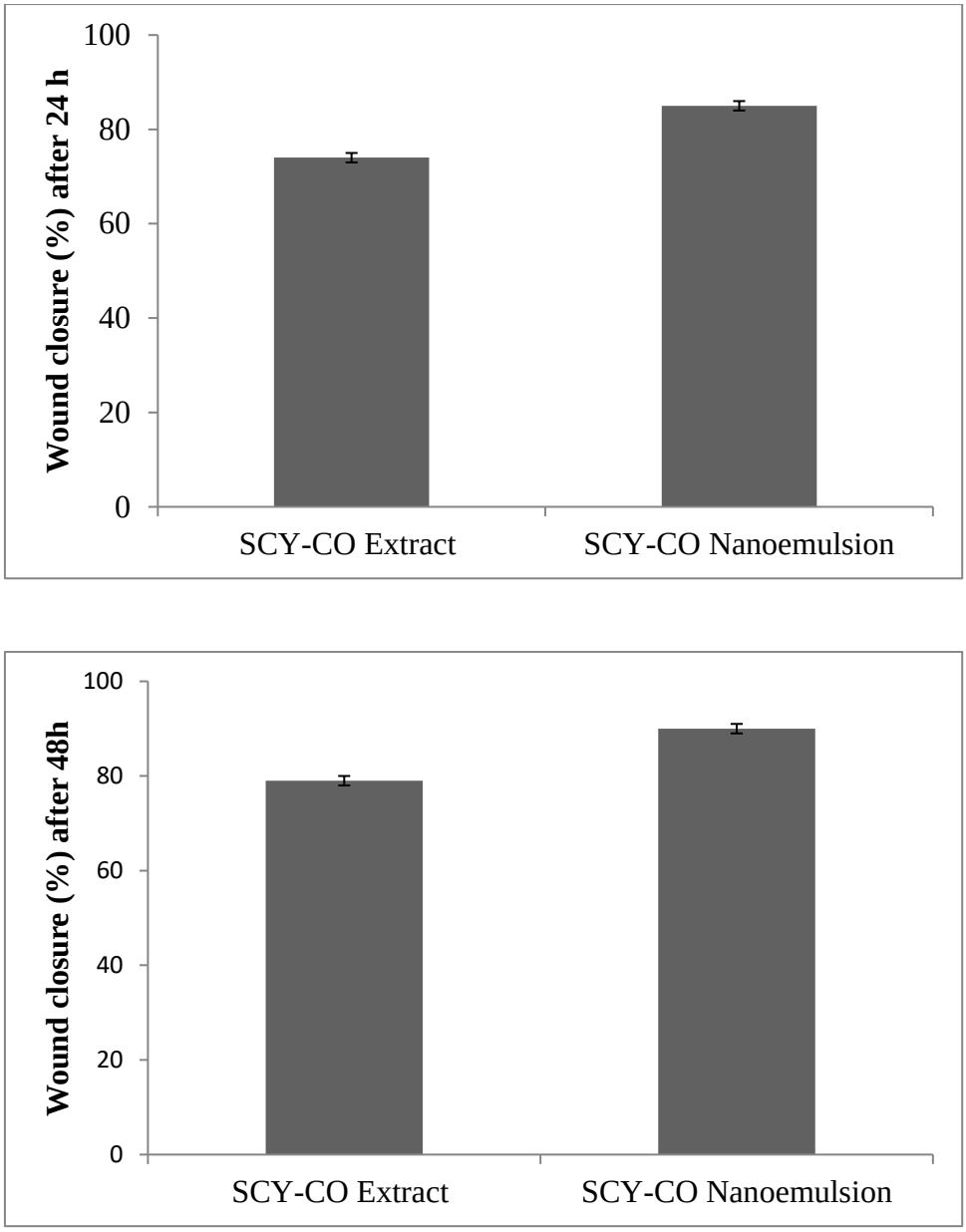


Fig. 11: Wound closures of HaCaT free gaps after 24h and 48h treatment with scytonemin –*Cydonia oblonga* extract and its loaded nanoemulsion. Data are expressed as % wound closure, ±SD, n = 6.

4.6.2 Determination of Intracellular ROS

ROS productions as seen under fluorescent microscope at the magnification of 20X. Treatment of HaCaT cell line with scytonemin-*Cydonia oblonga* extract and its loded nanoemulsion for 24 and 48 hr at 92(µg/ml induced ROS intracellular level. Reactive oxygen species (ROS) play a pivotal role in the orchestration of the normal wound-healing response. They act as secondary messengers to many immunocytes and non-

lymphoid cells, which are involved in the repair process, and appear to be important in coordinating the recruitment of lymphoid cells to the wound site and effective tissue repair. ROS also possess the ability to regulate the formation of blood vessels (angiogenesis) at the wound site and the optimal perfusion of blood into the wound-healing area. ROS act in the host's defence through phagocytes that induce an ROS burst onto the pathogens present in wounds, leading to their destruction, and during this period, excess ROS leakage into the surrounding environment has further bacteriostatic effects. In light of these important roles of ROS in wound healing and the continued quest for therapeutic strategies to treat wounds in general and chronic wounds, such as diabetic foot ulcers, venous and arterial leg ulcers and pressure ulcers in particular, the manipulation of ROS represents a promising avenue for improving wound-healing responses when they are stalled (Dunnill *et al*., 2017).

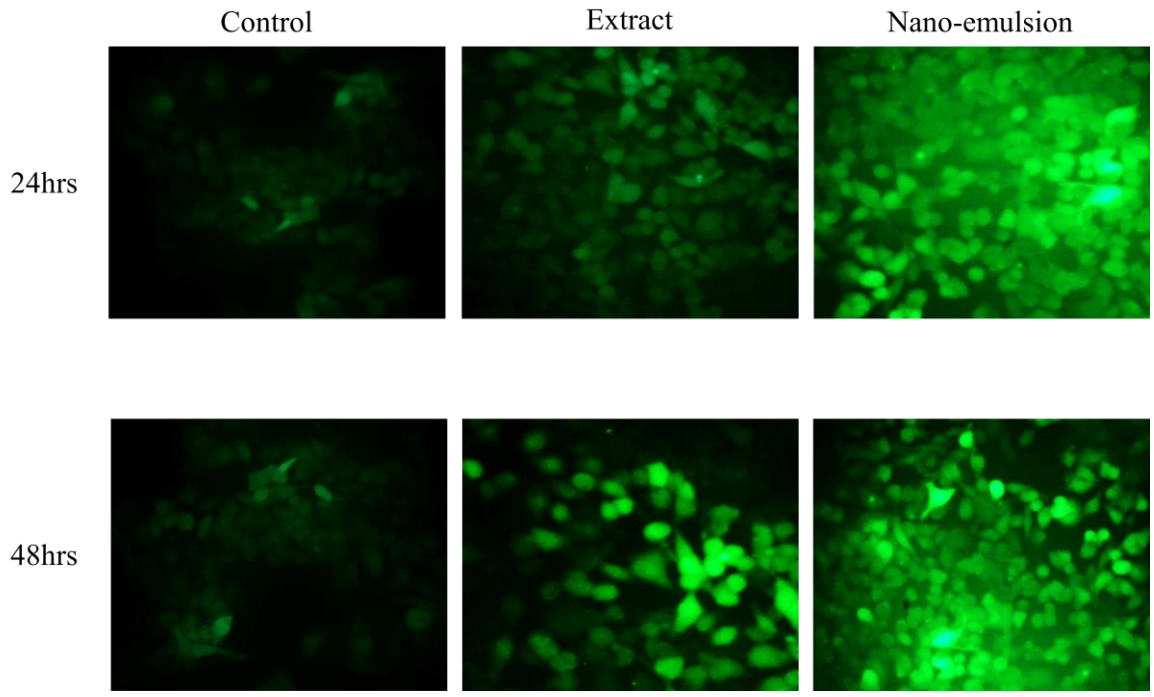


Fig. 12: Effect of Scytonemin-*Cydonia oblonga* extract and Scytonemin-*Cydonia oblonga* nanoemulsion with DCFHDA dye at IC_{50} value for 24hrs and 48hrs, respectively.

4.6.3 Cell Migration Assay

As a complex process aiming at restoring the function of skin against the environment, wound healing is accompanied with phases of reepithelialization involving proliferation and migration of keratinocytes. Transwell migration assay at 24hr showed that all the

Scytonemin–*Cydonia oblonga* loaded nanoemulsion enhances the migration capacity of HaCaT cells as compared to Scytonemin–*Cydonia oblonga* extract. In particular, in HaCaT cells treated for 24hr with Scytonemin *Cydonia oblonga* extract loaded nanoemulsion and Scytonemin–*Cydonia oblonga* extract there were about 94% and 75% of migrated cells respectively. Collective cell migration is a hallmark of wound repair, cancer invasion and metastasis, immune responses, angiogenesis, and embryonic morphogenesis. Wound healing is a complex cellular and biochemical process necessary to restore structurally damaged tissue. It involves dynamic interactions and crosstalk between various cell types, interaction with extracellular matrix molecules, and regulated production of soluble mediators and cytokines. In cutaneous wound healing, skin cells migrate from the wound edges into the wound to restore skin integrity. Analysis of cell migration in vitro is a useful assay to quantify alterations in cell migratory capacity in response to experimental manipulations. Although several methods exist to study cell migration (such as Boyden chamber assay, barrier assays, and microfluidics-based assays), in this short report we will explain the wound healing assay, also known as the "*in vit*ro scratch assay" as a simple, versatile, and cost-effective method to study collective cell migration and wound healing (Grada *et al*., 2017).

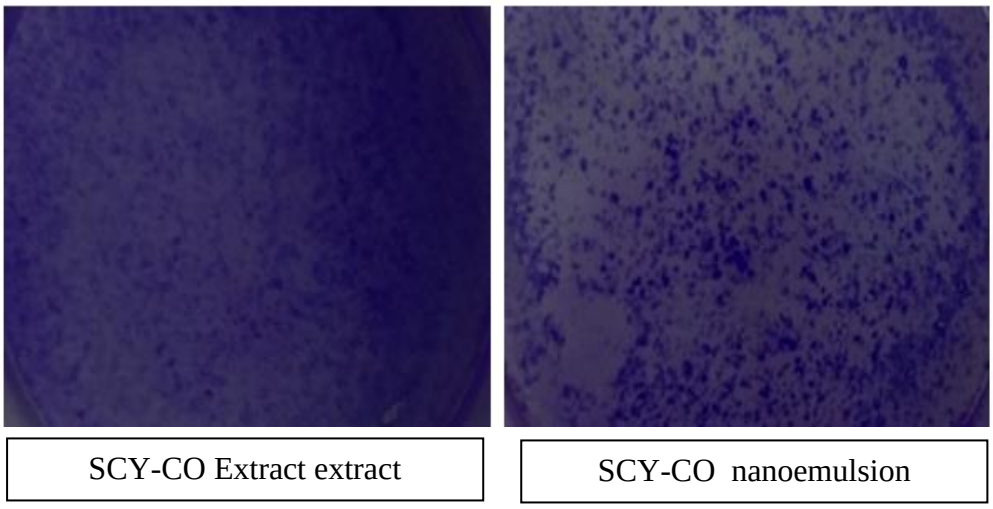


Fig. 13: Scytonemin–*Cydonia oblonga* nanoemulsion increased keratinocyte migration and proliferation. Representative images of the transwell migration assay upon treatment of keratinocytes with *Scytonemin-Cydonia oblonga* extract and its loded nanoemulsion (magnification × 20).

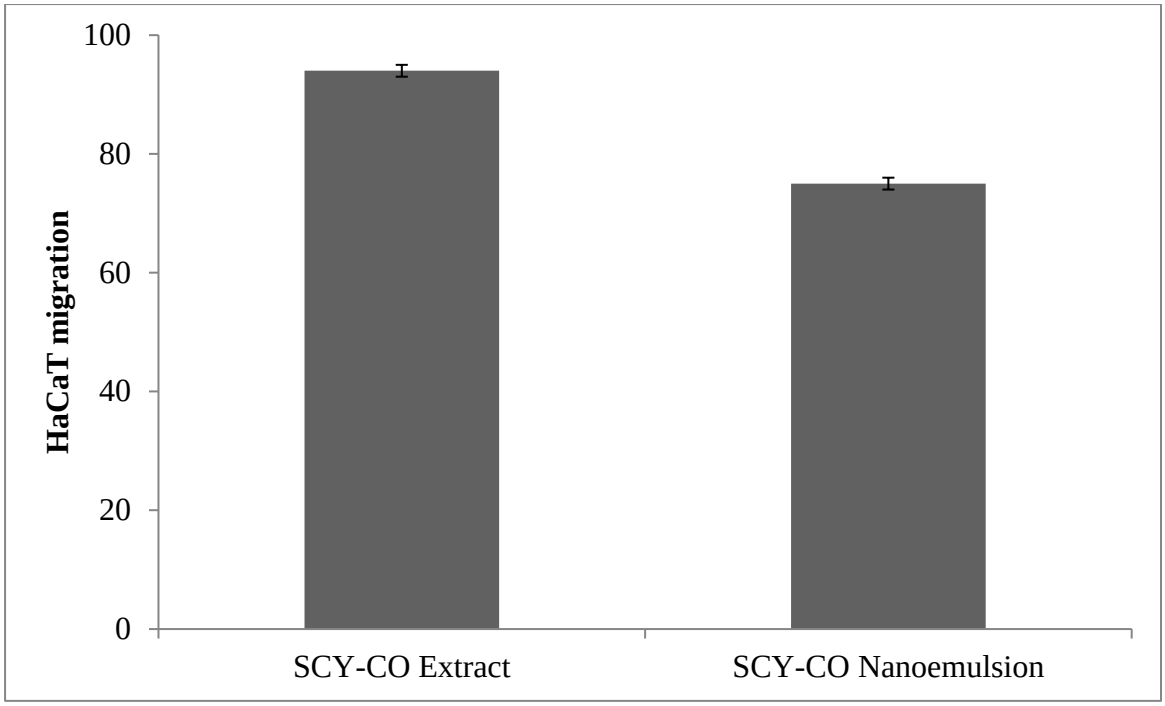


Fig. 14: Migration ability of keratinocytes represented as the percentage of cells penetrating the membrane.

5. CONCLUSIONS

Cydonia oblonga and *scytonema javanicum* extracts screened in this study exhibited good wound healing activity, a promising prospect for the development of wound healing agents. Coupled with the excellent antioxidant activities displayed by some of the extracts in this study using different mechanistic models, the good wound healing activities makes excellent combination that assist in various processes of inflammation. The extracts screened in this study are utilised by traditional healers as management recipes or natural resource for healing wounds and skin disorders. The mode of action for the dermatological use of medicinal plants is sometimes determined by their phenolic content, positive anti-inflammatory, antioxidant activity and protein interaction properties. Nanoemulsion was developed comprising the extracts of the medicinal plants including *Cydonia oblonga* and *Scytonema javanicum.* Anti-cancer, anti-inflammatory, anti-microbial, anti-hypertensive, and other properties have been documented for the medicinal plants listed above. The seeds of each plant were extracted in their individual solvents, and the extracts were then blended in an optimum ratio to create a scytonemin-*Cydonia oblonga* mixture. Scytonemin-*Cydonia oblonga* extracts was incorporated into a nanoemulsion (NE) by using the titration method to determine the percentage of Smix (surfactant and co-surfactant) and oil (Sefsol 218). The three phases of the NE were optimized using the pseudo ternary phase diagram (water, Smix and oil). The filtered ratios' thermodynamic stability was explored further. The size, shape, and stability of the selected ratios were evaluated further. Results of particle size, PDI, in vitro release suggested that scytonemin-*Cydonia oblonga* nanoemulsion was effective in inhibiting the proliferation of HaCaT cell lines. Overall, the study results do present some valuable anti-inflammatory and antioxidant activities of the selected medicinal plants that can be exploited for the healthcare benefit of humankind. . The moderate to good protein binding capacity exhibited by some of the tested medicinal plants could be used as a predictive wound healing model for these medicinal plants. According to their IC50 values, the generated nanoemulsion demonstrated a non-toxic effect. Fluorescence dye was used to measure the formulation's ROS potential and nuclear fragmentation activity.In comparison to the extract, the produced NE has a significantly higher capability for suppressing HaCaT cells. Moreover; scytonemin-*Cydonia oblonga* nanoemulsion was found to be stable for the longer period of time when compared with other formulations. As ROS altering compounds may have multiple roles in cancer, further detailed

investigations are warranted for its utility as anti-cancer agent. Scytonemin-*Cydonia oblonga* nanoemulsion showed higher anticancer activity of *in vitro* against HaCaT cell line. Once isolated the active compounds can further be used to determine and examine the mechanism of activity. These data, taken together, suggest that a future medication delivery method of scytonemin-*Cydonia oblonga* nanoemulsion extract loaded NE have the valuable capacity and potential to be used in wound healing and skin care management. Our findings show that understanding of existing medicinal systems can be utilised to bio prospect, select, develop, and market novel sources of medicines that are safe, cost-effective, and highly efficient, with superior targeted drug delivery capabilities.

6. REFERENCES

Abliz, A., Yimin, W., Zhou, W., Imam, G., Happar, M., Tohti, I., & Umar, A. (2012). Experimental study on antihypertensive effect of Cydonia oblongaoblonga Mill. *J. Xinjiang Medical University*, *35*(4), 432-435.

Abolghasem, J., Mohammadali, M. S., Mohammad, R. A., Maryam, K., Alireza, S., Paul, S. A., & Kamyar, G. (2011). The effect of quince leaf decoction on renal injury induced by hypercholesterolemia in rabbits: a pilot study. *Journal of medicinal plants Research*, *5*(21), 5291-5295.

Akhter, S., Jain, G. K., Ahmad, F. J., Khar, R. K., Jain, N., Khan, Z. I., & Talegaonkar, S. (2008). Investigation of nanoemulsion system for transdermal delivery of domperidone: ex-vivo and in vivo studies. *Current Nanoscience*, *4*(4), 381-390.

Alam, P., Shakeel, F., Anwer, M. K., Foudah, A. I., & Alqarni, M. H. (2018). Wound healing study of eucalyptus essential oil containing nanoemulsion in rat model. *Journal of oleo science*, ess18005.

Alonso, J. L., & Goldmann, W. H. (2003). Feeling the forces: atomic force microscopy in cell biology. *Life sciences*, *72*(23), 2553-2560.

Al-Snafi, A. E. (2016). The medical importance of Cydonia oblongaoblonga-A review. *IOSR Journal of Pharmacy*, *6*(6), 87-99.

Al-Snafi, A. E. (2022). Blood lipids lowering effect of medicinal plants.

Ashrafi, H., Ghabili, K., Alihemmati, A., Jouyban, A., Shoja, M. M., Aslanabadi, S., & Hajhosseini, L. (2013). The effect of quince leaf (Cydonia oblongaoblonga Miller) decoction on testes in hypercholesterolemic rabbits: a pilot study. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(2), 277-282.

Aslam, M., & Sial, A. A. (2014). Effect of hydroalcoholic extract of Cydonia oblongaoblonga miller (Quince) on sexual behaviour of wistar rats. *Advances in pharmacological sciences*, *2014*.

Balan, G., Bubalo, N., Bubalo, V., Zhminko, P., Nedopytanska, N., & Babich, V. (2017). Family of nuclear peroxisome proliferator—activated receptors (PPARs): biological role in metabolic adaptation. Part III. PPARγ and its role in autophagy, inflammation, carcinogenesis and reproductive system upon the exposure to xenobiotics (report 2). *Сучасні проблеми токсикології, харчової та хімічної безпеки*, (1-2), 65-79.

Bilal, M., & Iqbal, H. M. (2020). New insights on unique features and role of nanostructured materials in cosmetics. *Cosmetics*, *7*(2), 24.

Bolaji, B. O., & Huan, Z. (2013). Ozone depletion and global warming: Case for the use of natural refrigerant–a review. *Renewable and Sustainable Energy Reviews*, *18*, 49-54.

Bornman, J. F., Barnes, P. W., Robinson, S. A., Ballare, C. L., Flint, S. D., & Caldwell, M. M. (2015). Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems. *Photochemical & Photobiological Sciences*, *14*(1), 88-107.

Cheng, J., Park, J. H., Karimi, H. R., & Shen, H. (2017). A flexible terminal approach to sampled-data exponentially synchronization of Markovian neural networks with time-varying delayed signals. *IEEE Transactions on Cybernetics*, *48*(8), 2232-2244.

Church, B. (2013). *Medicinal Plants, Trees & Shrubs of Appalachia*. Tate Publishing.

Costa, R. M., Magalhães, A. S., Pereira, J. A., Andrade, P. B., Valentão, P., Carvalho, M., & Silva, B. M. (2009). Evaluation of free radical-scavenging and antihemolytic activities of quince (Cydonia oblongaoblonga) leaf: a comparative study with green tea (Camellia sinensis). *Food and Chemical toxicology*, *47*(4), 860-865.

Darvishi, E., Kahrizi, D., Arkan, E., Hosseinabadi, S., & Nematpour, N. (2021). Preparation of bio-nano bandage from quince seed mucilage/ZnO nanoparticles and its application for the treatment of burn. *Journal of Molecular Liquids*, *339*, 116598.

Derikvand, P., Llewellyn, C. A., & Purton, S. (2017). Cyanobacterial metabolites as a source of sunscreens and moisturizers: A comparison with current synthetic compounds. *European Journal of Phycology*, *52*(1), 43-56.

Duerst, M. (2007). Spectroscopic methods of analysis: infrared spectroscopy. *Swarbrick J., Boylon JC, Encyclopedia of Pharmaceutical Technology*, *3*, 3405-3418.

Dunnill, C., Patton, T., Brennan, J., Barrett, J., Dryden, M., Cooke, J., Leaper, D. and Georgopoulos, N.T., 2017. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *International wound journal*, *14*(1), pp.89-96.

Duscher, D., Maan, Z. N., Wong, V. W., Rennert, R. C., Januszyk, M., Rodrigues, M., & Gurtner, G. C. (2014). Mechanotransduction and fibrosis. *Journal of biomechanics*, *47*(9), 1997-2005.

Dwivedi, S., & Ahmad, I. Z. (2022). A review of the emerging role of cyanobacteria-based nanoformulations for skin care: Opportunities and challenges. *Journal of Applied Biology and Biotechnology*, *10*(3), 2-1.

Fazeenah, A. A., & Quamri, M. A. (2016). Behidana (Cydonia oblongaoblonga Miller)–a review. *World J Pharm Res*, *5*(11), 79-91.

Fernandes, D. (2005). Minimally invasive percutaneous collagen induction. *Oral and Maxillofacial Surgery Clinics*, *17*(1), 51-63.

Gao, Q., & Garcia-Pichel, F. (2011). Microbial ultraviolet sunscreens. *Nature Reviews Microbiology*, *9*(11), 791-802.

Garcia-Pichel, F., & Castenholz, R. W. (1991). Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment 1. *Journal of Phycology*, *27*(3), 395-409.

Gefen, A. (Ed.). (2019). Innovations and Emerging Technologies in Wound Care.

Ghafourian, M., Tamri, P., & Hemmati, A. (2015). Enhancement of human skin fibroblasts proliferation as a result of treating with quince seed mucilage. *Jundishapur Journal of Natural Pharmaceutical Products*, *10*(1).

Gilbert, M. E., Kirker, K. R., Gray, S. D., Ward, P. D., Szakacs, J. G., Prestwich, G. D., & Orlandi, R. R. (2004). Chondroitin sulfate hydrogel and wound healing in rabbit maxillary sinus mucosa. *The Laryngoscope*, *114*(8), 1406-1409.

Grada, A., Otero-Vinas, M., Prieto-Castrillo, F., Obagi, Z. and Falanga, V., 2017. Research techniques made simple: analysis of collective cell migration using the wound healing assay. *Journal of Investigative Dermatology*, *137*(2), pp.e11-e16.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C., & Omanyuda, M. (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (PseudoCydonia oblongasinensis Schneid.), quince (Cydonia oblongaoblonga Mill.), and apple (Malus domestica Mill.) fruits. *Journal of Agricultural and Food Chemistry*, *53*(4), 928-934.

Harborne, J. B. (1999). Classes and functions of secondary products from plants. *Chemicals from plants*, *26*, 1-25.

Hseu, Y. C., Chou, C. W., Kumar, K. S., Fu, K. T., Wang, H. M., Hsu, & Yang, H. L. (2012). Ellagic acid protects human keratinocyte (HaCaT) cells against UVA-induced oxidative stress and apoptosis through the upregulation of the HO-1 and Nrf-2 antioxidant genes. *Food and Chemical Toxicology*, *50*(5), 1245-1255.

Huber, R., Stintzing, F. C., Briemle, D., Beckmann, C., Meyer, U., & Gründemann, C. (2012). In vitro antiallergic effects of aqueous fermented preparations from Citrus and Cydonia oblongafruits. *Planta medica*, *78*(04), 334-340.

Kale, S. N., Kitture, R., Ghosh, S., Chopade, B. A., & Yakhmi, J. V. (2017). Nanomaterials as enhanced antimicrobial agent/activity-enhancer for transdermal applications: a review. *Antimicrobial Nanoarchitectonics*, 279-321.

Karegar-Borzi, H., Salehi, M., & Rahimi, R. (2016). Laūq: A Sustained-Release Dosage Form for Respiratory Disorders in Traditional Persian Medicine. *Journal of Evidence-Based Complementary & Alternative Medicine*, *21*(1), 63-70.

Karimi, A., Movahhed, M., HajiMehdipoor, H., & Allahyari, F. PHARMACEUTICAL SCIENCES.

Khademi, F., Danesh, B., Nejad, D. M., & Rad, J. S. (2013). The comparative effects of atorvastatin and quince leaf extract on atherosclerosis. *Iranian Red Crescent Medical Journal*, *15*(8), 639.

Khalifa, S. A., Shedid, E. S., Saied, E. M., Jassbi, A. R., Jamebozorgi, F. H., Rateb, M. E., & El-Seedi, H. R. (2021). Cyanobacteria—From the oceans to the potential biotechnological and biomedical applications. *Marine Drugs*, *19*(5), 241.

Khan, E., & Ahmad, I. Z. (2021). An insight into the prophylactic and therapeutic activities of golden apple (Cydonia oblongaoblonga Mill.) for the future cancer care and prevention: A review. *Annals of Phytomedicine*, *10*(2), 22-35.

Kumar, S., Nehra, M., Dilbaghi, N., Marrazza, G., Hassan, A. A., & Kim, K. H. (2019). Nano-based smart pesticide formulations: Emerging opportunities for agriculture. *Journal of Controlled Release*, *294*, 131-153.

Mast, B. A., Diegelmann, R. F., Krummel, T. M., & Cohen, I. K. (1992). Scarless wound healing in the mammalian fetus. *Surg Gynecol Obstet*, *174*(5), 441-451.

Metcalfe, A. D., & Ferguson, M. W. (2007). Bioengineering skin using mechanisms of regeneration and repair. *Biomaterials*, *28*(34), 5100-5113.

Minaiyan, M., Ghannadi, A., Etemad, M., & Mahzouni, P. (2012). A study of the effects of Cydonia oblongaoblonga Miller (Quince) on TNBS-induced ulcerative colitis in rats. *Research in pharmaceutical sciences*, *7*(2), 103.

Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced drug delivery reviews*, *54*, S131-S155.

Mushir, S., Deep, S., Fatma, T., & Student, P. G. (2014). Screening of cyanobacterial strains for UV screening compound scytonemin-environmental perspectives. *Screening*, *3*(2).

Osman, A. G., Koutb, M., & Sayed, A. E. D. H. (2010). Use of hematological parameters to assess the efficiency of quince (Cydonia oblongaoblonga Miller) leaf extract in alleviation of the effect of ultraviolet–A radiation on African catfish Clarias gariepinus (Burchell, 1822). *Journal of Photochemistry and Photobiology B: Biology*, *99*(1), 1-8.

Patel, R. P., & Joshi, J. R. (2012). An overview on nanoemulsion: a novel approach. *International Journal of Pharmaceutical Sciences and Research*, *3*(12), 4640.

Patel, S., Maheshwari, A., & Chandra, A. (2016). Biomarkers for wound healing and their evaluation. *Journal of wound care*, *25*(1), 46-55.

Pattanaik, B., Schumann, R., & Karsten, U. (2007). Effects of ultraviolet radiation on cyanobacteria and their protective mechanisms. In *Algae and cyanobacteria in extreme environments* (pp. 29-45). Springer, Dordrecht.

Quesada, A., Mouget, J. L., & Vincent, W. F. (1995). Growth of Antarctic cyanobacteria under ultraviolet radiation: UVA counteracts UVB inhibition 1. *Journal of Phycology*, *31*(2), 242-248.

Ramanunny, A. K., Wadhwa, S., Gulati, M., Singh, S. K., Kapoor, B., Dureja, H., & Pandey, N. K. (2021). Nanocarriers for treatment of dermatological diseases: principle, perspective and practices. *European journal of pharmacology*, *890*, 173691.

Rastogi, R. P., Sinha, R. P., Moh, S. H., Lee, T. K., Kottuparambil, S., Kim, Y. J., & Han, T. (2014). Ultraviolet radiation and cyanobacteria. *Journal of Photochemistry and Photobiology B: Biology*, *141*, 154-169.

Rippka, R. O. S. E. M. A. R. I. E. (1972). Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. *Archiv für Mikrobiologie*, *87*(1), 93-98.

Sadeghi Mahoonak, A., Ghorbani, M., Kashani Nejad, M., & Maghsoudlou, Y. (2017). Optimization of Ultrasound-assisted extraction of Quince seed mucilage using

Response Surface Methodology. *Journal of Food Processing and Preservation*, *9*(2), 17-31.

Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K., & Longaker, M. T. (2009). Human skin wounds: a major and snowballing threat to public health and the economy. *Wound repair and regeneration*, *17*(6), 763-771.

Shinomiya, F., Hamauzu, Y., & Kawahara, T. (2009). Anti-allergic effect of a hot-water extract of quince (Cydonia oblongaoblonga). *Bioscience, biotechnology, and biochemistry*, *73*(8), 1773-1778.

Silva, B. M., Andrade, P. B., Valentão, P., Ferreres, F., Seabra, R. M., & Ferreira, M. A. (2004). Quince (Cydonia oblongaoblonga Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. *Journal of Agricultural and Food Chemistry*, *52*(15), 4705-4712.

Singer, A. J., & Clark, R. A. (1999). Cutaneous wound healing. *New England journal of medicine*, *341*(10), 738-746.

Singh, R. P., Sharad, S., & Kapur, S. (2004). Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. *J Indian Acad Clin Med*, *5*(3), 218-225.

Souto, W. M., Mourão, J. S., Barboza, R. R. D., & Alves, R. R. (2011). Parallels between zootherapeutic practices in ethnoveterinary and human complementary medicine in northeastern Brazil. *Journal of ethnopharmacology*, *134*(3), 753-767.

Tamjidi, F., Shahedi, M., Varshosaz, J., & Nasirpour, A. (2013). Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. *Innovative Food Science & Emerging Technologies*, *19*, 29-43.

Tansaz, M., Akhtari, E., Bioos, T., & Mokaberinejad, R. (2013). The efficacy of Cydonia oblongaoblonga extract on infant's reflux: a pilot study

Vasconcelos, A., & Cavaco-Paulo, A. (2011). Wound dressings for a proteolytic-rich environment. *Applied microbiology and biotechnology*, *90*(2), 445-460.

Vater, C., Bosch, L., Mitter, A., Göls, T., Seiser, S., Heiss, & Klang, V. (2022). Lecithin-based nanoemulsions of traditional herbal wound healing agents and their effect on human skin cells. *European Journal of Pharmaceutics and Biopharmaceutics*, *170*, 1-9.

Visheratina, A. K., Purcell-Milton, F., Gun'ko, Y. K., & Orlova, A. (2019). Circular dichroism spectroscopy as a powerful tool for unraveling assembly of chiral nonluminescent aggregates of photosensitizer molecules on nanoparticle surfaces. *The Journal of Physical Chemistry A, 123*(37), 8028-8035.

Walmsley, G. G., Maan, Z. N., Wong, V. W., Duscher, D., Hu, M. S., Zielins, E. R., & Longaker, M. T. (2015). Scarless wound healing: chasing the holy grail. *Plastic and reconstructive surgery*, *135*(3), 907-917.

Zhang, L., & Webster, T. J. (2009). Nanotechnology and nanomaterials: promises for improved tissue regeneration. *Nano today*, *4*(1), 66-80.

Zhou, W., Abdusalam, E., Abliz, P., Reyim, N., Tian, S., Aji, & Umar, A. (2014). Effect of Cydonia oblongaoblonga Mill. fruit and leaf extracts on blood pressure and blood rheology in renal hypertensive rats. *Journal of Ethnopharmacology*, *152*(3), 464-469.