ESTIMATING THE WOUND HEALING POTENTIAL OF SUPERCRITICALLY EXTRACTED CURCUMIN (CUR-95%) AND TURMERIC OIL (TUR-60%) IN L929 FIBROBLAST CELL LINE

A DISSERTATION SUBMITTED TO ST. TERESA'S COLLEGE (AUTONOMOUS), ERNAKULAM IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF

DEGREE OF MASTER OF SCIENCE IN ZOOLOGY

SUBMITTED BY

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CERTIFICATE

This is to certify that the dissertation entitled **' Estimating the wound healing potential of super-critically extracted Curcumin (CUR-95%) and Turmeric oil (60%) in L929 Fibroblast cell line'** submitted to St. Teresa's College (Autonomous), Ernakulam, in partial fulfilment of the requirement of award of degree of Master of Science in Zoology is an authentic work carried out by **Ms. VISHNU PRIYA M G** (SM20ZOO011) in the academic year 2020 – 2022 under the guidance and supervision of **Dr. P Sreejith** (External Guide) Assistant Professor, Department of Zoology, University of Kerala, Karyavattom Campus, Thiruvananthapuram and **Mrs. Indu Vasudevan** (Internal Guide), Assistant Professor, Department of Zoology, St. Teresa's College, Ernakulam.

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DECLARATION

I hereby declare that the dissertation entitled '**Estimating the wound healing potential of Supercritically extracted Curcumin (CUR-95%) and Turmeric oil (60%) in L929 Fibroblast cell line**' submitted to St. Teresa's College (Autonomous), Ernakulam in partial fulfilment of the requirements, for the award of the Degree of Master of Science in Zoology is a record of original research work done by me under the supervision and guidance of **Dr. P Sreejith** (External Guide) Assistant Professor, Department of Zoology, University of Kerala, Karyavattom Campus, Thiruvananthapuram during the period from 1st April 2022 – 30th April 2022 and **Mrs. Indu Vasudevan** (Internal Guide), Assistant Professor, Department of Zoology, St. Teresa's College, Ernakulam, to the best of my knowledge and belief, this project contains no material previously published or written by another person, except where due reference is made.

VISHNU PRIYA M G

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LIST OF ABBREVIATIONS

SL. NO.	ABBREVIATION	EXPLANATION
1.	%	Percentage
2.	µg	Microgram
3.	mm	Millimetre
4.	°C	Degree celsius
5.	hr	Hour
6.	mL	Millilitre
7.	µL	Microlitre
8.	µm	Micrometre
9.	g	Gram
10.	ng	Nanogram
11.	pH	Potential Hydrogen
12.	RT	Room temperature
13.	rpm	Rotation per minute
14.	mins	Minute
15.	Vol.	Volume
16.	DNA	Deoxyribonucleic acid

17.	FBS	Fasting Blood Sugar
18.	DMSO	Dimethyl sulfoxide
19.	qPCR	Quantitative polymerase chain reaction
20.	ddH_2O	Double distilled water
21.	RNA	Ribonucleic acid
22.	PCR	Polymerase chain reaction
23.	DMEM	Dulbecco's Modified Eagle medium
24.	PBS	Phosphate buffered saline
25.	NaCl	Sodium chloride
26.	KCl	Potassium chloride
27.	MMP-13	Matrix metalloproteinase 13
28.	ECM	Extracellular matrix
29.	qRTPCR	Real- Time Quantitative Reverse Transcription PCR
30.	DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
31.	ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
32.	MAPK	Mitogen-activated protein kinase
33.	TNFα	Tumor necrosis factor alpha

34.	IL	Interleukin
35.	MIP	Macrophage inflammatory protein
36.	MCP	Monocyte chemotactic protein
37.	CRP	C reactive protein
38.	PGE	Prostaglandin E
39.	COX	Cyclooxygenase
40.	NF-Kβ	Nuclear factor kappa B
41.	$Ca(OCl)_2$	Calcium oxychloride
42.	NaOCl	Sodium hypochlorite
43.	CHX	Chlorhexidine
44.	ANOVA	Analysis of Variance
45.	hPDL	4-Hydroxyphenylpyruvate Dioxygenase like
46.	OCT	Optical coherence tomography
47.	Na_2HPO_4	Sodium Phosphate
48.	KH_2PO_4	Monopotassium phosphate
49.	hEGF	Human Epidermal Growth Factor
50.	NCTC	National Collection of Type Cultures
51.	BME	Basal Medium Eagle
52.	IU	International Unit

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ABSTRACT

Wound healing implicates several biological and molecular events, such as coagulation, inflammation, migration-proliferation, and remodelling. This study aims to evaluate the effect of Supercritical Extracted Turmeric Oil with more than 60% Turmerones and 95% Curcumin Powder on wound healing conducted on Fibroblast cell line. On conducting the scratch assay, the optimal concentration of curcumin and turmeric oil were found to be 6.25µg and 3.12µg respectively. One of the most important factors in wound healing process are Fibronectin and MMP-13 gene. Fibronectin is essential for tissue formation in both wound repair and connective tissue repair and one of the building blocks in extracellular matrix formation. It will interacts with other cells to form an ECM in all stages of wound healing. In the case of Matrix metalloproteinase (MMP-13) they play a pivotal role, with their inhibitors, in regulating extracellular matrix degradation and deposition that is essential for wound re-epithelialization. By conducting q RT-PCR process, the concentration of curcumin results in the up regulation of Fibronectin and the concentration of turmeric resulted in down regulation when compared with Control and MMP-13 shows that, Curcumin leads to the up regulation of MMP-13 and turmeric leads to down regulation as compared to control. In conclusion, it has been found that curcumin possesses powerful modulating effects on wound healing than that of turmeric. Curcumin does this by acting on the inflammatory, proliferative and remodeling phases of the wound healing process and in doing so, reduces the time needed for wound healing.

INTRODUCTION

Medicinal plants have been used in healthcare since time immemorial. Studies have been carried out globally to verify their efficacy and it leads to the production of plant-based medicines (Sofowora et al. 2013). Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat diseases. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years. Medicinal plants based traditional systems of medicines are playing important role in providing health care to large section of population, especially in developing countries (Yuan et al. 2016).

India, one of the biodiversity-rich tropical countries, contributes about 7% of the world's biodiversity. Medicinal plants are found from Himalayan to Marine and Desert to Rain forest ecosystems. Out of the 17,000-18,000 species of flowering plants, more than 7,000 are estimated to have a usage in folk medicine and documented medicine systems like Ayurveda, Unani, Siddha, and Homeopathy (AYUSH System of Medicine). Millions of Indians regularly use herbal medicines in spices, health foods, home remedies, and self-medication. The people of India from time immemorial have been using thousands of medicinal plants for curing various diseases suggesting that Ayurveda has been widely used to maintain human health (Bahadur et al. 2005).

Standardization of medicinal plants ensure their consistency and therapeutic effectiveness. Herbal medicines have the potential to treat and cure illnesses like ulcers, healing of wounds, skin infections inflammation, scabies, leprosy, and venereal disease. Herbal medicines in wound treatment or care include disinfection, debridement, and providing a moist atmosphere which facilitates development of appropriate natural healing climate. Folklore cultures employ a significant number of plants to treat cuts, wounds, and burns (Sharma et al. 2021).

A wound is a disruption of living tissue’s cellular, anatomical, and functional integrity caused by physical, chemical, electrical, or microbial threats to the tissue. Skin wounds affect the quality of life of the patients significantly and are considered as one of major causes of physical disabilities. At the moment the available

treatments are suboptimal. Skin wounds are defined as any interruption of the continuity of body surface. They can be result of many conditions such as burns, surgeries, trauma, and arterial disease (Hosseinkhani et al. 2016). Wound healing is defined as a complex process occurring by regeneration or reconstruction of damaged tissue. The normal response to wound healing is a concerted sequence of events that begins with an injury. When platelets come into contact with exposed collagen, the healing cascade is initiated, causing the accumulation of platelets as well as the release of coagulating factors which in turn result in the formation of a fibrin clot at the injury site. The fibrin clot functions as a temporary matrix which sets the tone for activities that accompany healing. Inflammatory cells, along with the platelets provide essential signals known as cytokines or growth factors; also arrive at the injury site. The fibroblast is the connective tissue responsible for collagen deposition that is needed to fix tissue damage. Collagen provides strength, integrity, and structure in normal tissues. Collagen is required to repair the defect and restore anatomical structure and function when tissues are damaged after injury. If healing does not progress stepwise in the usual way, then it can lead to chronic growth of wounds. Medicinal plants such as *Curcuma longa (L.), Terminalia arjuna, Centella asiatica, Bidens Pilosa, Aloe barbadensis,* and *Rauwolfia* serpentine have confirmed wound healing activity (Sharma et al. 2021).

Certain compounds like tannins could promote wound healing through free radical removal, increasing the contraction of the affected area and increasing the formation of blood vessels and fibroblasts. Other active principles such as triterpenes, alkaloids, and flavonoids have proven to be effective in this process of wound healing (Hosseinkhani et al. 2016).

Many plants regulated the skin's natural healing process and therefore have enormous possibilities for therapeutic use in wound care. They employed commonly used scientific methodology to study plants and their extracts from the physiological and pharmacological point of view, along with an increase in familiarity with herbal extracts and isolates, the numberof herbal products for wound treatment is increasing steadily. Clinical pieces of evidence of the therapeutic effects of herbal products have led to the study of many more herbs for their therapeutic, either curative or preventative, roles. Such a combination of modern and traditional knowledge can develop novel drugs for wound healing with significantly lowered side effects.In the

primary health care of 80% of the world's underdeveloped and developing countries are played by a natural source of medicinal treatment and plant-based system. Natural agents regulate healing and tissue regeneration through multiple coupled mechanisms (Maver et al. 2015).

Out of 250,000 flowering plant species in the world, 15% have been approximated phytochemically, and only 6% have been checked for biological activity. A comparatively small portion of all plants have been used as medicinal agents; their significance should not be undermined as almost 65% of the world's population has integrated them into their primary modality of healthcare. Compared to modern drugs, about one-third of all traditional herbal medicines are intended for the treatment of wounds or skin disorders. In the United States 6.5 million patients were affected by chronic wounds, and that the number of chronic woundsare expected to grow worldwide due to the increase in age-related conditions and pathologies such as diabetes, obesity, and cardiovascular diseases.

Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies. Because of its brilliant yellow color, turmeric is also known as "Indian saffron." More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil, containing turmerone, and there are other coloring agents called curcuminoids in turmeric. Curcuminoids consist of curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidant. In folk medicine, turmeric has been used in therapeutic preparations over the centuries in different parts of the world. In Ayurvedic practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones, and relieving arthritis. It can also be used in antiseptic for cuts, burns, and bruises, and act as an antibacterial, anti-inflammatory agent, and as a remedy for gastrointestinal discomfort associated with irritable bowel syndrome and other digestive disorders (Prasad et al. 2011).

Curcumin, the most active component of rhizome of *Curcuma longa L*., has been studied for many years due to its bio-functional properties, especially antioxidant,

radical scavenger, antimicrobial and anti-inflammatory activities, which play a crucial role in the wound healing process. Moreover, curcumin stimulated the production of the growth factors involved in the wound healing process, and so curcumin also accelerated the management of wound restoration (Tejada et al. 2016).

Turmeric was described as *C. longa* by Linnaeus, and its taxonomic position is as follows:

Kingdom : Plantae

Clade : Tracheophytes

Clade : Angiosperms

Clade : Monocots

Clade : Commelinids

Order : Zingiberales

Family : Zingiberaceae

Genus : *Curcuma*

Species : *longa*

The wild turmeric is called *Curcuma aromatic*, and the domestic species is called *Curcuma longa* (Chattopadhyay et al. 2004).

Figure 1: Showing Turmeric rhizome

(*https://uvicpermaculture.files.wordpress.com/2016/05/termer.jpg?w=423&h=317*)

Fibroblasts are critical in supporting normal wound healing, involved in key processes such as breaking down the fibrin clot, creating new extra cellular matrix (ECM) and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound. These cells are responsible for tissue homeostasis under normal physiological conditions. When tissues are injured, fibroblasts become activated and differentiate into myofibroblasts, which generate large contractions and actively produce extracellular matrix (ECM) proteins to facilitate wound closure. Both fibroblasts and myofibroblasts play a critical role in wound healing by generating traction and contractile forces, respectively, to enhance wound contraction (Li et al. 2011).

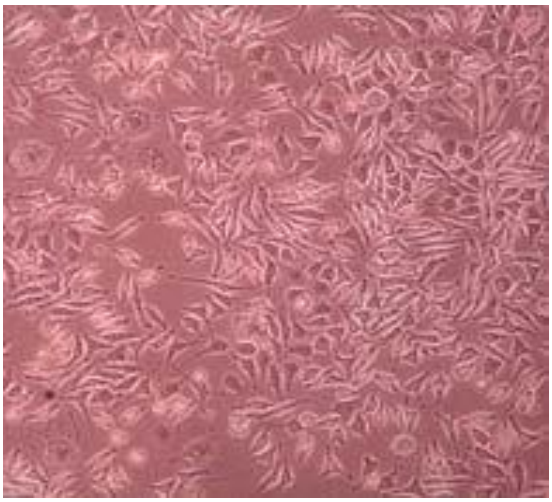

Figure 2: Showing L929 mouse fibroblast cell line

https://www.researchgate.net/topics

In the present study, the wound healing potential of Curcumin and Tumeric oil, is being explored in mouse fibroblast cell line and we used **Supercritical Extracted Turmeric Oil with more than 60% Turmerones** and **95% Curcumin Powder. Supercritical fluid extraction** is the process in which by using supercritical fluids as the extracting solvent, one component is separated fromanother. The most commonly used supercritical fluid is CO_2 and also modified co-solvent such as ethanol and methanol are used. The optimal condition for this process is the critical temperature of 31^0C and a critical pressure of 74 bar. Already many studies are carried out to find the medicinal and healing efficiency of turmeric and antioxidant, anti-angiogenesis

property of curcumin. However, here in this study, many properties of both compounds are examined simultaneously.

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AIM AND OBJECTIVE

AIM:

The aim of this study is to examine the efficiency of Supercritically extracted Curcumin and Turmeric oil on artificially created wound in L929 mouse fibroblast cell line. The result obtained from scratch assay is validated through qRT-PCR analysis.

OBJECTIVE:

- Estimating the wound healing potential of super-critically extracted Curcumin and Turmeric oil in Fibroblast cell line.
- To validate the results using q RT-PCR analysis.

RELEVANCE OF THE STUDY:

The present study enable to make people aware of plant products and their health benefits in organisms and their influential role in wound healing and the revelation of new treatments, protocols, and procedures. Some of these new treatments mainly rely on the development of novel biomaterials with antibacterial and anti-oxidant properties for wound dressing, promoting an accelerated healing.

REVIEW OF LITERATURE

The study conducted by Steiner et al. (2021) aims on the significance of collagen in different biological processes relevant to wound healing. Collagen, a key component of the extracellular matrix, plays critical roles in the regulation of the phases of wound healing either in its native, fibrillar conformation or as soluble components in the wound milieu. In response to injury, collagen induces platelet activation and aggregation resulting in the deposition of a fibrin clot at the injury site. The type, amount and organization of collagen changes in the healing wound and determines the tensile strength of the healed skin. Collagen III is the first to be synthesized in the early stages of wound healing and is replaced by collagen I, the dominant skin collagen. Collagen contributes to the mechanical strength and elasticity of tissues and acts as a natural substrate for cellular attachment, proliferation, and differentiation. Collagen degradation is involved in inflammation, angiogenesis, and re-epithelialization in the wound regulated by complex molecular pathways. During the inflammation phase, soluble fragments from collagen degradation recruit immune cells such as macrophages that patrol the wound for removal of microbes and devitalized tissue. This aids in the transition to the proliferative phase. During this stage, collagen fragments serve as potent angiogenic signals to promote the development of new blood vessels. Collagen exposure due to injury activates the clotting cascade, resulting in a fibrin clot that stops the initial bleeding. Collagen I and IV fragments can be mediators of inflammation by acting as potent chemoattractants for neutrophils, enhancing phagocytosis and immune responses and modulating gene expression. Inflammation is a critical step in the normal process of wound healing and drives the proliferation of fibroblasts which synthesize collagen and ECM.

The study directed by Chittasupho et al. (2021) on the Human Dermal Fibroblast cells evaluated the effect of the combination of quercetin and curcuminoids at three different ratios on the antimicrobial, antioxidant, cell migration and wound healing properties. The antioxidant activities of quercetin, curcuminoids and the mixtures were tested by DPPH and ABTS free radical scavenging assays. The cytotoxicity and cell migratory enhancing effects of quercetin, curcuminoids and the mixtures against human dermal fibroblasts were investigated by MTT assay, scratch assay and

Transwell migration assay, respectively. The results showed the synergism of the quercetin and curcuminoid combination to inhibit the growth of S. aureus and P. aeruginosa, with the inhibition zone ranging from 7.06-0.25 to 8.78-0.38 mm, respectively. Quercetin and a 3:1 quercetin/curcuminoid mixture at non-toxic concentrations showed the ability to stimulate the migration of fibroblasts across the matrix, whereas only quercetin alone accelerated the wound closure of fibroblasts. In conclusion, the mixture of quercetin and curcuminoids at a 3:1 ratio was the best formulations for use in wound healing due to the antimicrobial, antioxidant and cell-migration-enhancing activities.

An *in vivo* study was investigated by Adeliana et al. (2021) on female rabbit (*Oryctolagus cuniculus*) by the application of (*Curcuma Longa Linn*) Gel Extract. The purpose of this study was to identify differences in the length of the wound at each con-centration of gel preparations on days 3, 7, and 14, as well as differences in wound healing time at each concentration of gel preparations and, identify the most effective gel preparations for wound healing. The sample size in this study was 12 rabbits grouped randomly. The length of each group's wounds was measured and observed on days 3, 7, and 14. Gel application was carried out twice a day in the morning and evening for 14 days. In this study, the experimental data were tested using Kruskal Wallis. Based on the mean wound length of each group, they experienced a reduction in wound length on days 3, 7, and 14. There were also differences in wound healing time in each group. In each group, the treatment group that was given 5% turmeric extract gel experienced a faster healing time <14 days than the other groups. In general, turmeric extract gel at each concentration is effective against wound healing. The low concentration in this study is the concentration of turmeric extract gel 5%, considered more effective in providing a wound-healing effect in rabbits, then followed by concentrations of 10% and 15%.

In the study conducted by Udayakumar et al. (2020), cold percolated ethanol leaf extracts of *Beta vulgaris* and *Psidium guajava* were compared for its *in vitro* wound healing activity through scratch wound assay performed on L929 cells. The rate of healing was examined at regular intervals and determined using Image J software. This study aims to compare the *in vitro* wound healing activity of the selected ethanol extracts with that of the standard positive control, thereby extending its

application for *in vivo* wound healing capacity useful in the cosmetic industries. The results shows that the selected ethanol leaf extracts of beetroot and guava were added to the culture well plates in differing concentrations like 25µg, 50µg, 75µg and 100µg along with the standard positive control. Beetroot showed increased cell proliferation on the created wound (wound area – 3331µm) at a minimum concentration of 25µg with the maximum wound closure of 78% after 24 hours. At increasing concentrations they displayed lesser healing activity due to the increased levels of ethanol suppressing the healing activity of beetroot. Guava showed a lesser healing capacity on the created wound. Similar to the beetroot extracts, guava also showed a decreasing linearity over its healing potential due to the inhibition of ethanol against epithelial cell proliferation. On the whole, both the selected plant extracts were found to exert comparable wound healing effects with the positive control.

The study conducted by Kaptaner Igci & Aytaç Z, (2020) investigated the *in vitro* wound healing effects of the methanolic and aqueous extracts of *Hypericum pseudolaeve* .Total phenolic and flavonoid contents were measured using spectrophotometry-based methods. The cytotoxic effects of the extracts on L929 mouse fibroblast cells were evaluated by and 2h-tetrazolium,5 -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Moreover, migration and spreading of the treated fibroblast cells were assessed by cell scratch assay as an *in vitro* wound healing model. The results of the cytotoxicity assay indicated that the methanolic and aqueous extract did not have any cytotoxic effect on fibroblast cells at concentrations up to 500 µg/mL. Fibroblast migration was significantly increased by 62 µg/mL concentration of the aqueous extracts. The results showed that *Hypericum pseudolaeve* extracts have wound healing potential and contain several important antioxidant phenolic compounds.

The study conducted by Sharifi-Rad et al. (2020) deals with the *in vitro* and *in vivo* studies on curcumin and it has shown that a great potential for treating inflammatory diseases. It was shown that curcumin can inhibit pro-inflammatory transcription factors (NF-kB and AP-1) and the mitogen activated protein kinases (MAPK) and pathways involved in nitric oxide synthase (NOS) enzymes synthesis; Reduce the proinflammatory cytokines TNFa, IL-1b, IL-2, IL-6, IL-8, MIP-1a,

MCP-1, CRP, and PGE2; Down-regulate enzymes such as 5- lipoxygenase and COX-2 and -5.The anti-inflammatory mechanisms are shown in curcumin is also able to modulate the NF-kB expression. In fact, the NF-kB pathway activation leads to proinflammatory cytokine production, such as interleukin (IL-1, IL-2, IL-6, IL-8) and TNFa. It could decrease the oxidative stress and inflammation through the Nrf2 pathway.

Through his study Dandannavar et al. (2019) evaluated the wound-healing potential of the plant extract on mouse fibroblast cell line L929 through scratch assay and the potential effect of the plant extract on the expression of two key wound-healing factors, fibronectin and collagen 1 was also studied. In this study L929 was exposed to various concentrations of *R. korthalsii* methanol extract to check the cytotoxic effect of the extract. *In vitro* scratch assay was performed on the treated cells to assess the wound-healing ability of the extract. Further, the effect of the extract on the expression of wound-healing factors, fibronectin and collagen 1, was studied through a flow cytometric analysis. *R. korthalsii* leaf extract showed no toxicity on the L929 fibroblast cells. The treated cells showed ~93% wound closure after 48 h of incubation. The treatment of cells with the leaf extract, also, upregulated the expression of the wound-healing factors, fibronectin and collagen 1, i.e., 76.30% and 96.09% of the cells expressed them, respectively. The results shows that *R. korthalsii* is a non-toxic plant with wound-healing properties. The phytochemicals in this plant may be responsible for its abilities. Thus, it can serve as an alternative therapy against synthetic drugs for wound healing.

The study evaluated by Llerena et al. (2019) deals with the cytotoxicity and cell migration of calcium hypochlorite [$Ca(OCl)_2$] and octenidine hydrochloride - OCT (OcteniseptR, Schulke & Mayr, Norderstedt, Germany) in L929 and human periodontal ligament (hPDL) cells. The cells were exposed to different doses of different solutions: 2.5% and 5% $Ca(OCl)_2$, 0.1% OCT, 2.5% NaOCl and 2% CHX for 10 min. Cell viability was assessed by methyl-thiazol-tetrazolium (MTT) and neutral red (NR) assays, and cell migration was determined by wound-healing assay. Statistical analysis was performed by two-way ANOVA and Bonferroni tests (α=0.05). The MTT and NR assays revealed that 0.1% OCT was less cytotoxic in hPDL cells ($p<0.05$), followed by 2% CHX and 2.5% $Ca(OCl)_2$ ($p<0.05$). There was

no significant difference between 2.5% NaOCl and 5% $Ca(OCl)_2$ ($p>0.05$), but these solutions showed greater cytotoxicity than the others. The result was the same for L929 cells, except that there was no significant difference between 2% CHX and 2.5% $Ca(OCl)_2$ ($p>0.05$). Wound-healing assay in L929 and hPDL cells showed that cell migration of 0.1% OCT, 2% CHX and 2.5% $Ca(OCl)_2$ groups was higher than 5% $Ca(OCl)_2$ and 2.5% NaOCl groups at 24 h.In the result it has shown that, 0.1% OCT had lower cytotoxicity in tested cell line than CHX, $Ca(OCl)_2$ and NaOCl. Cell migration was higher for 0.1% OCT, 2% CHX and 2.5% $Ca(OCl)_2$. Therefore, in terms of cytotoxicity, OCT and $Ca(OCl)_2$ have the potential to be used as root canal irrigants.

The study directed by Bolla et al. (2019) was aimed at investigating the wound healing capacity of *Aristolochia saccata* leaf extract by using scratch assay as a primary model, where proliferative and migratory capabilities of test compounds could be monitored through microscopy studies. *A. saccata* is an evergreen climbing shrub belonging to the family Aristolochiaceae .Here, methanolic extraction of the plant material was done using Soxhlet apparatus and the cytotoxicity of the extract on L929 cells was studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *In vitro* scratch assay was performed to evaluate the wound healing properties of *A. saccata* leaf extract and possible mechanism of action was analyzed by flow cytometric expression studies of an extracellular matrix (ECM) factor, collagen type-1. MTT assay revealed that *A. saccata* leaf extract had no cytotoxic effect on the cells and at higher concentrations, the extract showed mild toxicity resulting in the death of just 2.88% cells. Scratch assay showed 4.05%, 70.00%, 93.52% wound closure at 12hrs, 24hrs and 48hrs of incubation respectively. These results were similar compared to positive control which showed 37.60, 56.41 and 99.05% of wound closure. Further, flow cytometry-based studies revealed that the *A. saccata* leaf extract induced the expression of ECM remodelling factor collagen-1.Finally,the study revealed the wound healing capabilities of *A. saccata in vitro*. Hence, they found that *A. saccata* could be recommended as a potential source of wound healing agents.

The study conducted by Borges et al. (2017), aimed to evaluate ozone cytotoxicity in fibroblasts (L929) and keratinocytes (HaCaT) cell line, its effects on cell migration and its antimicrobial activity. Cells were treated with ozonated phosphate-buffered saline (8, 4, 2, 1, 0.5 and 0.25 mg/mL ozone), chlorhexidine 0.2% or buffered-solution, and cell viability was determined through MTT assay. The effect of ozone on cell migration was evaluated through scratch wound healing and transwell migration assays. The minimum inhibitory concentrations for *Candida albicans* and *Staphylococcus aureus* were determined. Ozone showed no cytotoxicity for the cell line, while chlorhexidine markedly reduced cell viability. Although no significant difference between control and ozonetreated cells was observed in the scratch assay, a considerable increase in fibroblasts migration was noticed on cells treated with 8 mg/mL ozonated solution. Ozone alone did not inhibit growth of microorganisms; however, its association with chlorhexidine resulted in antimicrobial activity. This study confirms the wound healing and antimicrobial potential of ozone therapy and presents the need for studies to elucidate the molecular mechanisms through which it exerts such biological effects.

The effects of topical curcumin was studied by Emiroglu et al. (2017) on the healing of nasal mucosal wounds. A total of 32 Sprague-Dawley Albino rats were randomized in equal numbers into four groups, and unilateral nasal wounds were created using an interdental brush. Group 1 (the sham-control group) contained untreated rats with traumatized right-side nasal cavities; Group 2 and 3 rats were similarly traumatized and treated with topical curcumin (5 and 10 mg/mL) dissolved in dimethyl sulfoxide daily for 7 days after trauma; Group 4 rats were treated with topical dimethyl sulfoxide only. All rats were decapitated on day 15 and the healing sites evaluated by blinded observers in terms of the presence of cellular hyperplasia, goblet cell hypertrophy and degeneration, leucocytic infiltration, ciliary loss and degeneration, edema, and vascular dilation. On histopathological evaluation, all of cellular hyperplasia, leukocytic infiltration, and edema were significantly reduced in Group 3 compared with Group 1. The results shows that curcumin reduced the inflammatory response and significantly accelerated wound healing.

The study conducted by Toden et al. (2017) on the essential turmeric oils enhance anti-inflammatory efficacy of curcumin in dextran sulfate sodium induced colitis,

reveals the anti-inflammatory efficacy and associated gene expression alterations of a specific, curcumin preparation containing essential turmeric oils (ETO-curcumin) in comparison to standard curcumin at three specific doses (0, 5, 25 or 50 mg/kg), in an animal model of dextran sodium sulfate (DSS)-induced colitis. The present study showed that both ETO and standard curcumin treatments provided protection against DSS-induced inflammation. However, ETO-curcumin improved disease activity index (DAI) dose-dependently, while the anti-inflammatory efficacy of standard curcumin remained constant, suggesting that ETO-curcumin may provide superior anti-inflammatory efficacy compared to standard curcumin. Gene expression analysis revealed that anti-inflammatory cytokines including IL-10 and IL-11 as well as FOXP3 were upregulated in the colon by ETO-curcumin. Collectively, these findings suggest that the combined treatment of curcumin and essential turmeric oils provides superior protection from DSS-induced colitis than curcumin alone, highlighting the anti-inflammatory potential of turmeric.

Human wound healing studies was conducted by Nuutila et al. (2013) describes the usefulness and limitations of some of the techniques for the characterization of human wound healing in a clinical setting. Novel techniques have enabled high-end molecular level research even from small samples of tissue. Since these methods require only a small amount of patient skin, they make it possible to study wound healing directly in humans. With techniques such as the polymerase chain reaction (PCR), microarray, and RNA-sequencing (RNA-seq), it is possible to investigate how different genes' expressions are altered in skin during trauma or disease. More importantly, these techniques offer an unbiased approach to the observation of healing. Understanding the basic mechanisms behind the wound-healing cascade and then discovering means to regulate them for faster healing or to avoid negative outcomes such as infection or scarring is very important to wound research.

The study evaluated by Velnar et al. (2009) aims on wound classification, the physiology of the wound healing process and the methods used in wound management**.** Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines. Although the process of healing is continuous, it may be arbitrarily divided into four phases: coagulation and haemostasis; inflammation; proliferation; and wound

remodelling with scar tissue formation. The correct approach to wound management may effectively influence the clinical outcome.

The study investigated by Fronza et al. (2009) deals with the optimization of the scratch assay which can be used as an in vitro model for quantification of fibroblast migration to and proliferation into the wounded area. It is suitable for the first evaluation of the wound re-epithelialization potential of crude herbal extracts, isolated compounds and pharmaceutical preparations. As a proof of concept three preparations from traditional medicinal plants were investigated. Swiss 3T3 albino mouse fibroblasts were used in monolayers and platelet derived growth factor as positive control. Hexane and ethanolic extracts from *Calendula officinalis* and *Matricaria recutita*, Hypericum oil as well as the triterpenoids faradiol myristate and palmitate were studied. To differentiate between proliferation and migration antimitotic mitomycin C was added. The results shows that both extracts of *Calendula officinalis* stimulated proliferation and migration of fibroblasts at low concentrations, 10 g/ml enhanced cell numbers by 64.35% and 70.53%, respectively. Inhibition of proliferation showed that this effect is mainly due to stimulation of migration. Faradiol myristate and palmitate gave comparable stimulation rates at an almost 50g/ml concentration, indicating that they contribute partially, but not most significantly to the wound healing effects of Calendula preparations. Extracts from *Matricaria recutita* were only moderately active. Hypericum oil was cytotoxic at concentrations higher than 0.5 g/ml. This study shows that scratch assay in the present form can be used as a promising scientific approach and platform to differentiate between plant extracts known for their wound healing and their anti-inflammatory properties.

MATERIALS AND METHODS

In the present study, the biological efficiency of supercritically extracted Turmeric oil which containing more than 60% turmerone and 95% Curcumin powder were analysed on L929 mouse fibroblast cell line. The medicinal properties of turmeric are well known, but yet no studies were done in turmeric oil containing more than 60% turmerone, hence through this assay, the efficacy of turmerone was also evaluated. Regular turmeric that we consume contains only 2.5% of Curcumin. The activity of 95% supercritically extracted curcumin on wound healing was also analysed in this study.

COMPOUNDS USED FOR STUDY

Compounds are obtained from Akay Flavours & Aromatics Pvt. Ltd., Ernakulam.

- Supercritical Extracted Turmeric Oil with more than 60% Turmerone (TUR 01/17)
- 95% Curcumin Powder (CC R 01/17)

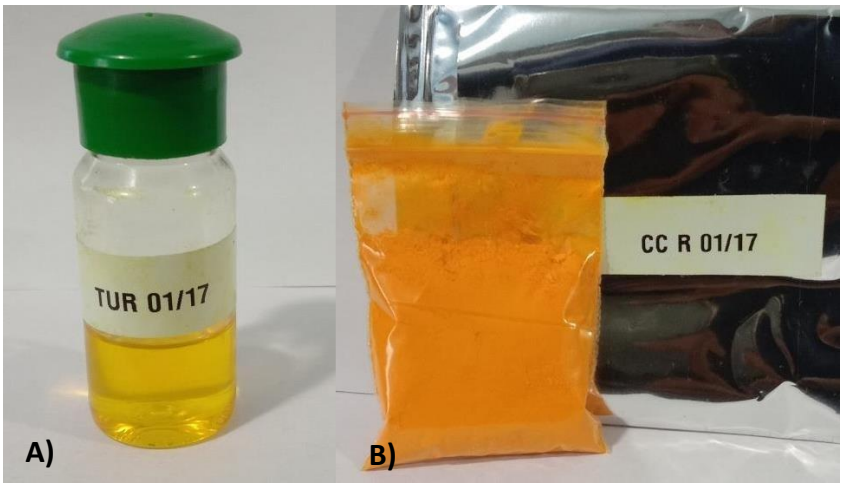

Figure 3: **A)** Supercritical Extracted Turmeric oil. **B)** 95% Curcumin Powder

CELL LINE USED FOR *IN-VITRO* STUDIES

The parent of fibrosarcoma cell line L929 is derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse, and they represent thus adult somatic-derived cells. This Adriamycin-resistant cell line has been developed by exposure of the parent L929 murine fibroblast cell line to increasing concentrations of doxorubicin in-vitro. Clone L929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain. NCTC clone 929 Clone of strain L (also known as L929) was one of the first cell strains to be established in continuous culture, and clone 929 was the first cloned strain developed. L929/A cells can be used in the development of novel anti-cancer treatments. Resistance can be circumvented by modulating agents such as verapamil and quinine (Cadagan et al. 2013).

CULTURE AND MAINTEINANCE OF CELL LINE

Dulbecco's Modified Eagle's Medium-low glucose:

Basal Medium Eagle (BME) is modified as Dulbecco's Modified Eagle medium (DMEM). It contains four-fold concentrations of the amino acids and vitamins, and the level of glucose has been raised to 4500mg/l in DMEM/High. To culture embryonic mouse cells, theoriginal formulation contained 1000 mg/L of glucose and was used. Since then, to support primary cultures of mouse cells, it has been modified in several ways as well as a variety of normal and transformed cells. Different combination of L-glutamine and sodium pyruvate were offered in each of these media.

Phosphate-Buffered Saline (10X) pH 7.4, RNase -free (PBS):

Phosphate buffer saline (PBS) is a pH-adjusted phosphate buffer, and saline solutions their osmolarity and ion concentrations of the solution usually match those of the human body (isotonic) and non-toxic to many of the cells. Attains 137 mM NaCl, 2.7

mM KCl, 8 Mm Na_2HPO_4 , and 2 mM KH_2PO_4 when diluted to a 1X working concentration. It is a salty solution containing potassium chloride, potassium phosphate and sodium phjosphate and sodium chloride. PBS is certified RNase-free tested rigorously for containg nonspecific endonuclease, exonuclease and RNase activity.

Trypsin:

Trypsin a most frequently used member of serine protease family to detach cell from the adherent substrate. From the proenzyme trypsinogen secreted by the exocrine cell of the pancreas, trypsin is produced, they mainly act on C-terminal side of lysine or arginine. Prewarmed trypsin speed up the detachment and optimum activity are achieved at 37ºC. Incubation with high trypsin concentration for a long time causes cell damage by striping cell surface proteins and kill the cells. Many cell types tolerate trypsin, but various concentration and constituents of trypsin are employed based on the application and type of cell.

Antibiotics:

The culture medium is supplemented with antibiotics to prevent fungal and bacterial infection. Antibiotics function by degrading the cell membrane of the infectious bacterium and fungus, respectively. A working concentration of 50-100 IU/ml penicillin and 50-100µg/ml Streptomycin. Pencillin-Streptomycin solution were widely used antibiotics in cell culture.

The Revival of Cell Line:

A Cryopreserved vial of cells was taken, allowed to thaw in the water bath at 37°C and transferred the whole content of vial into a sterile 15ml falcon tube and 2ml of media was added slowly within 5 min then it was allowed to centrifuge at 2000rpm for about 5mins. Afterthe centrifugation, pelleted cells have gently resuspended the cells using 5ml media and it was transferred into a T25 flask. These cells were incubated in 5% CO_2, 37° C incubator for attachment and further growth of the cell. The cells were grown without changing the medium until they were seen to be attached to the flask. After complete attachment of the cell,the media was changed.

Splitting and cell counting using Hemocytometer:

The growth of cells in culture progress from the **lag phase** following the **log phase**, where the exponential proliferation of cells occurs. When all the available substrates were occupied by the adherent cells, then there will be no space left for expansion. For the continued growth and to stimulate further proliferation, the culture has to divide, and new media were supplied to keep them at an optimal density. A decline in pH of the growth medium usually indicates the secretion of lactic acid, which is a by-product of cellular metabolism. The decrease in pH is harmful to cell growth, and lactic acid can be toxic to the cells. The faster exhaustion of the media occurs at high cell concentration than the lower cell concentrations; hence, the concentration of cells in those medium affects the rate of change of pH. After removing the media, PBS wash was given and then through the side of the flask trypsin was added after that the flask is incubated at room temperature for 2 mins to detach the cells. Afterthe detachment of 90% of cells, a pre-warmed complete medium was added to this flask to inhibit the action of trypsin. Cells were transferred into 15ml falcon tube and centrifuged at 2000 rpmfor 7 mins. The cell pellet was resuspended using media, and 10ul were taken for cell enumeration and to check the percentage of viability using hemocytometer after staining with trypan blue. The cells were counted in the four-field and average of the count was taken.

The volume of the cell suspension to be taken from the qualified cell suspension = Average no. of cells × Total vol. × Dilution Factor × 10^4

Dilution factor = 2

Cryopreservation of Cells:

To maintain the stock of cells, cell freezing and cryopreservation are very important. This is done when the cell in the flask becomes confluent. Trypsinize the fully confluent flaskand 10% of the media were added to stop the action of trypsin. Then centrifugation of the vial was carried out at 2000 rpm for 7 mins. The supernatant was discarded, and 700ml of DMSO and FBS mixed in 1:9 ratio (Freezing Medium)

were added to the pellet. Resuspend the cells thoroughly in the freezing medium. The cells were frozen in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute. Cells containing cryo vials are placed in an isopropanol chamber and store them at -80°C overnight. The frozen cells were transferred to liquid nitrogen, and store them in the gas phase above the liquid nitrogen (Freshney, 2015).

CHECKING THE CELL MIGRATION USING SCRATCH ASSAY

The wound healing study has been widely used to study the efficacy of test compounds on mammalian cell migration and proliferation.

In this assay, a cell free area is created in the confluent monolayer by physical exclusion or by removing the cells from the area using pipette tip. The healing processing is monitored by cell migration towards the gap that is been created. The gap width and rate of cell migration is monitored in zero hour, 24 hour and 48 hour.

Depending on the efficacy of the compounds the rate of migration and growth of cells can be monitored.

L929 were seeded into 12-well tissue culture plate, after 24 hr they reach 70-80% confluency. Without changing the medium, a scratch was made on the monolayer using 200 µl pipette tip. The detached cells were removed by giving PBS wash.The 12 well plate will be viewed under the inverted microscope in zero hour without adding the compounds. In every 24 hours, the gap was observed through the microscope until the wound gap get closed. The photographs were taken by using Leica software.

RNA ISOLATION (Trizol Method))

- Scrape the cells using Cell Scrapes in PBS.
- Centrifuge and discard the supernatant
- Add 500 µL Trizol.
- Homogenise using a homogenizer.
- Make the volume 1 ml using Trizol and vortex the mixture.
- Incubate at room temperature for 5 min.
- Add 200 µL of Chloroform added and mixed well by shaking.
- Incubate at room temperature for 10-15 min.
- Centrifuge the mixture at 13,000 rpm for 15 min at 4ºC.
- Collect the supernatant into afresh tube.
- Add 500 µL (ice cold) Isopropyl alcohol and mix gently.
- Incubate at room temperature for 10 min.
- Centrifuge at 13,000 rpm for 10 min at 4ºC.
- Discard the supernatant.
- Add 500 µL (ice cold) 70 ethanol to the pellet and centrifuge 13,000 rpm for 5 min at 4ºC.
- Discard the supernatant and air dry the pellet.
- Dissolve the air dry pellet to 100 µL RNase free water.

cDNA SYNTHESIS:

The volume of each component is for a 20µL final reaction:

Table 1: Showing the protocol for cDNA Synthesis

	Volume	**Final concentration**
5X cDNA synthesis buffer	4 µL	1X
dNTP Mix	2 µL	500µM
RNA Primer	1 µL	

RT Enhancer	1 µL	
Verso Enzyme Mix	1 µL	
Template (RNA)	1.5 µL	1ng
Water, nuclease-free (#R0581)	To 20 µL	
Total volume	20 µL	

q RTPCR:

1) Prepare qPCR reaction mixture:

a) Please prepare the q PCR reaction solution according to the list:
The following protocol is recommended for a 20 reaction volume:

Table 2: Showing the protocol for preparing qPCR reaction mixture

Components	**Volume**
2 AB HS SYBR Green qPCR Mix	10 µL
Forward Primer (2µM)	2 µL
Reverse Primer(2µM)	2 µL
DNA Template	2 µL
ddH_2O	4 µL
Final Volume	20 µL

b) After assembling all the components, gently mix the contents of the tube. Air bubbles may be generated during the mixing, to remove the bubbles apply low-speed

centrifugation.

2) Perform quantitative PCR

Perform quantitative PCR using optimized cycling conditions. Provided below is a standard two-step program.

Table 3: Showing thermocycling conditions for three-step qPCR

Step	Temperature	Time	Number of cycles	Fluorescence Detector
Intial denaturation	95 °C	2 min	1	Off
Dentauration	95 °C	10 sec	40	Off
Annealing	60 °C	20-30 sec	40	On

RESULTS

SCRATCH ASSAY- L929 CELL LINE

Curcumin Powder-95%

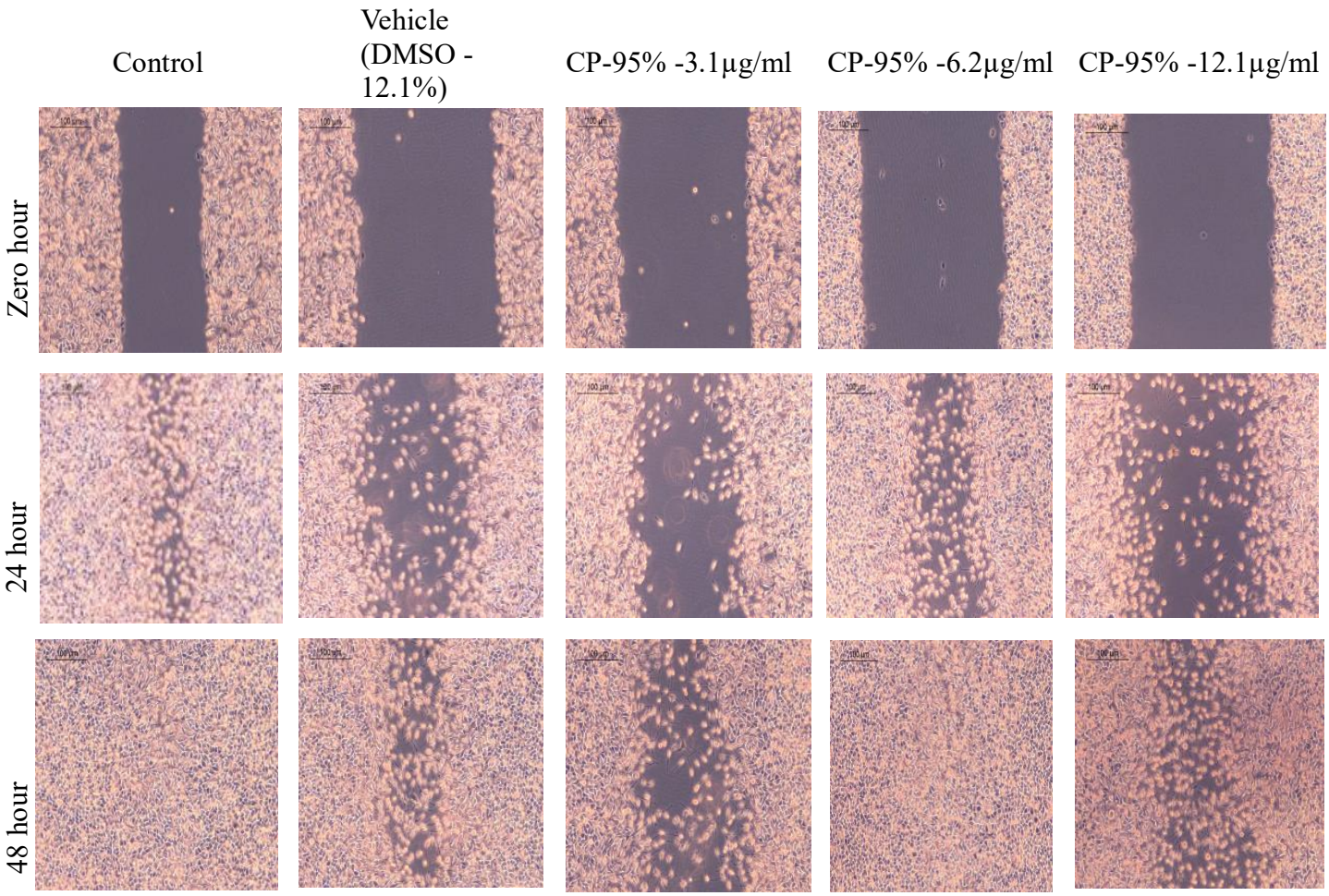

Figure 4: Microscopic image (10X) of L929 Cell migration after scratch at time 0, 24h and 48h of Curcumin treatment.

SCRATCH ASSAY- L929 CELL LINE

Supercritical Extracted Turmeric Oil (SE-Turmeric oil)

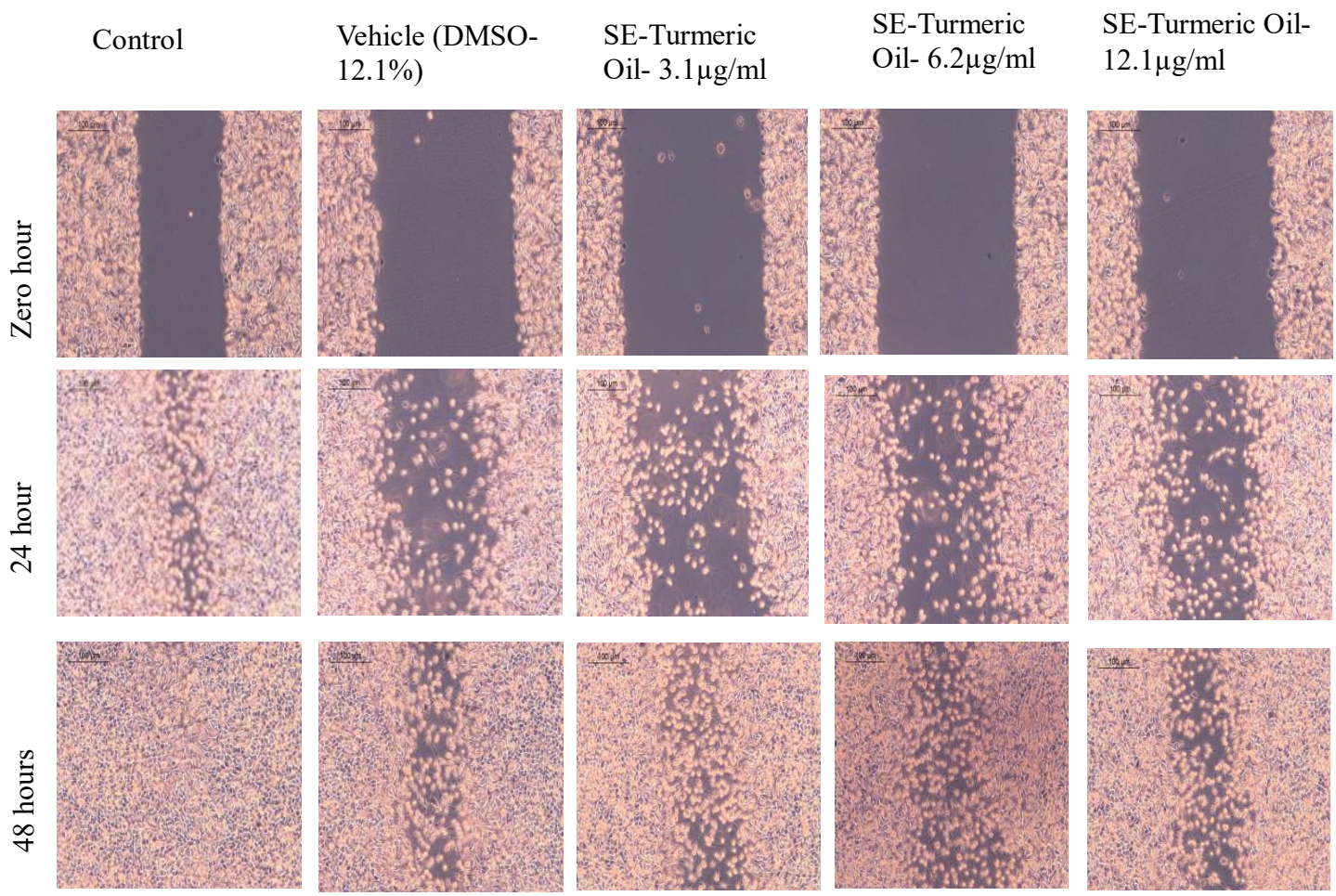

Figure 5: Microscopic image (10X) of L929 Cell migration after scratch at time 0, 24h and 48h of Turmeric oil treatment.

SCRATCH ASSAY- L929 CELL LINE

The wound-healing assay is simple, inexpensive, and one of the earliest developed methods to study directional cell migration *in vitro* (Rodriguez et al., 2005). It is particularly suitable for studies on the effects of cell-matrix and cell-cell interactions on cell migration. After 48 hours of incubation, the L929 cell line treated with curcumin powder and Turmeric oil showed more cell migration property than control. When compared with control after 24hrs of treatment, cells are migrating perpendicular to the wound. The wound-healing properties of Curcumin Powder were assessed through *in vitro* scratch assay. There was a significant increase in the percentage of cell migration with time in the L929 that were treated with the test compounds. In the concentration 6.25 µg/ml (Figure 4: J, K, L) of supercritically extracted curcumin shows decrease in wound gap as compared with respective control and vehicle. In the case of comparing the concentration 3.125 µg/ml (Figure 5: G, H, I) of supercritically extracted turmeric oil shows increase in wound gap as compared with respective control and vehicle.

qRT-PCR for Gene Expression of Fibronectin and MMP-13

	Control	Curcumin 95%	Turmeric 60%
Fibronectin	1	52.09	14.03
	0.152	0.656	3.959
MMP-13	1	15.8	0.08
	0.380	7.540	0.355

Table 4: Shows gene expression of Fibronectin and MMP-13 on qRT-PCR analysis

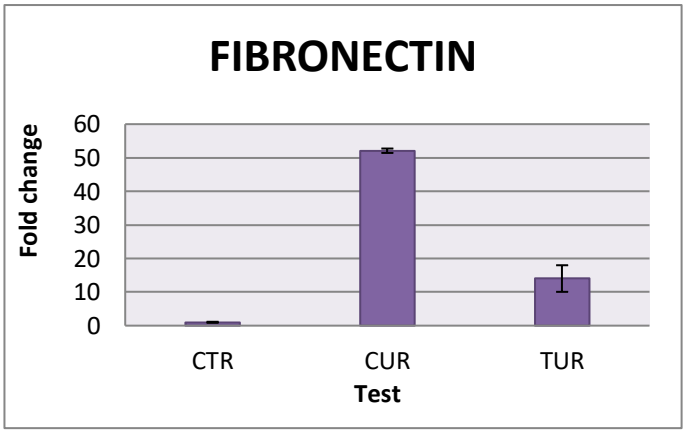

Figure 6: a

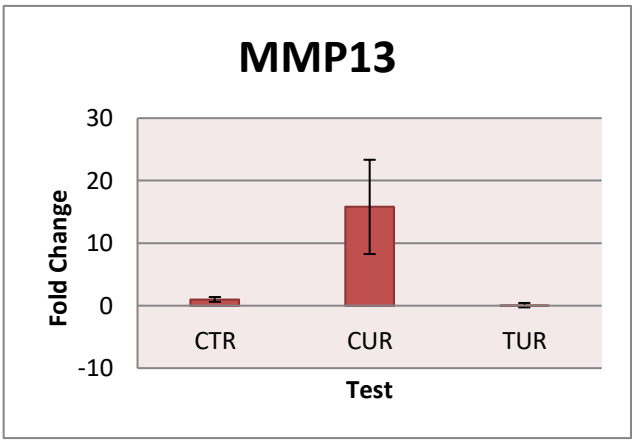

Figure 6: b

Figure 6: a) Graph showing test result of Fibronectin gene expression when treated with curcumin 6.25 µg/ml and turmeric 3.125 µg/ml. **b)** Graph showing test result of MMP-13 gene expression when treated with curcumin 6.25 µg/ml and turmeric 3.125 µg/ml

qRT-PCR for Gene Expression of Fibronectin and MMP-13

L929 cell line

Real-Time Quantitative Reverse Transcription (PCR) is a major development of PCR technology that enables reliable detection and measurement of products generated during each cycle of PCR process. The sufficient concentration that is obtained through the scratch assay were taken into consideration, where 6.25µg of Curcumin and 3.12µg of Turmeric oil shows increased cell migration to the wounded site. These concentration are further analysed through Real-Time Quantitative Reverse Transcription PCR.The gene expression of Fibronectin and MMP-13(Collagenase 3) were conducted. It has been clear from the Figure 6(a) that the concentration of Curcumin has resulted in the up regulation of the gene Fibronectin and the concentration of Turmeric has resulted in down regulation when compared with Control. On considering Figure 6(b) MMP-13, Curcumin leads to the up regulation of MMP-13 and turmeric leds to downregulation as compared to control.

DISCUSSION

Wound healing is a complex mechanism and a variety of plants used traditionally in folk medicine have been ethnopharmacologically validated for their wound healing properties. A number of *in vitro* studies have been conducted with the crude plant extracts or isolated secondary metabolites to understand their extended use in wound healing. Several therapeutic medicinal properties of curcumin have been discovered against rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, wound healing, urinary tract infections and liver ailments (Bolla et al., 2019). The plants belonging to the family Zingiberaceae have been reported both in traditional and modern medical seems to have antioxidant, anti-carcinogenic, anti-inflammatory, antimicrobial, deeming the plants to be medicinally relevant and important. Therefore, the present study evaluated the wound healing properties of super-critically extracted curcumin and turmeric oil on L929 fibroblast cell line. In order to assess the wound healing potency of the compounds, scratch assay, a widely used *in vitro* assay was performed in L929 fibroblast cell line. The L929 cells migrated in an accelerated rate towards the artificially created wounded region when treated with 95% curcumin than that of 60 % turmerones. This imply the fact that curcumin is more effective in wound healing than that of turmeric oil. An optimum concentration of 6.25µg/ml Curcumin and 3.12µg/ml Turmeric oil were taken into consideration and through q-RTPCR it is indicated that curcumin has resulted in the up regulation of Fibronectin and the concentration of turmeric results in down regulation when compared with Control. In the case of MMP-13, Curcumin results in up regulation and turmeric leads to downregulation as compared to control.

A similar study was conducted by Emiroglu et al., (2017) reveals the effects of topical curcumin on the healing of nasal mucosal wounds. A total of 32 Sprague-Dawley Albino rats were randomized in equal numbers into four groups, and unilateral nasal wounds were created using an interdental brush. Group 1 (the sham-control group) contained untreated rats with traumatized right-side nasal cavities; Group 2 and 3 rats were similarly traumatized and treated with topical curcumin (5 and 10 mg/mL) dissolved in dimethyl sulfoxide daily for 7 days after trauma; Group 4 rats were treated with topical dimethyl sulfoxide only. All rats were decapitated on day 15 and the healing sites evaluated by blinded observers in terms of the presence

of cellular hyperplasia, goblet cell hypertrophy and degeneration, leucocytic infiltration, ciliary loss and degeneration, edema, and vascular dilation. Thus, this study came to the conclusion that curcumin reduced the inflammatory response and significantly accelerated wound healing. This came to the conclusion that curcumin has the capacity to accelerate the wound closure in L929 fibroblast cell line and has high efficiency in wound closure at the concentration 6.25µg/ml rather than 3.12µg/ml and 12.1µg/ml respectively.

An in vivo study was investigated by Adeliana et al. (2021) on female rabbit (Oryctolagus cuniculus) by the application of (Curcuma Longa Linn) Gel Extract reveals that the turmeric extract gel at each concentration is effective against wound healing. The low concentration in their study is the concentration of turmeric extract gel 5%, considered more effective in providing a wound-healing effect in rabbits, then followed by concentrations of 10% and 15%. The present study reveals that, Turmeric oil is showing high efficiency in wound healing in L929 cell line at the concentration 3.12µg/ml rather than 6.25µg/ml and 12.1µg/ml respectively.

The study conducted by Toden et al. (2017) on the essential turmeric oils enhance anti-inflammatory efficacy of curcumin in dextran sulfate sodium induced colitis, reveals the anti-inflammatory efficacy and associated gene expression alterations of a specific, curcumin preparation containing essential turmeric oils (ETO-curcumin) in comparison to standard curcumin at three specific doses (0, 5, 25 or 50 mg/kg), in an animal model of dextran sodium sulfate (DSS)-induced colitis. This reveals that combined treatment of curcumin and essential turmeric oils provides superior protection from DSS-induced colitis than curcumin alone, highlighting the anti-inflammatory potential of turmeric. In present study, it has been revealed that suocurcumin (95%) shows high rate of migration of cells towards the artificially created wounded area rather than turmeric oil (60%).

The study conducted by Steiner et al. (2021) aims on the significance of collagen in different biological processes relevant to wound healing. Collagen, a key component of the extracellular matrix, plays critical roles in the regulation of the phases of wound healing either in its native, fibrillar conformation or as soluble components in the wound milieu. In response to injury, collagen induces platelet activation and aggregation resulting in the deposition of a fibrin clot at the injury site. In present

study, it has been analysed that curcumin leads to the up regulation of MMP-13 (Collagenase 3) and turmeric oil leads to the down regulation.

The study conducted by Dandannavar et al., (2019) reveals the wound-healing properties of the methanol extract of *R. korthalsii* leaves that was assessed through in vitro scratch assay. There was a significant increase in the percentage of cell migration with time in the L929 cells that were treated with the methanolic extract of *R. korthalsii* leaves. The wound healing properties of the methanolic extract of *R. korthalsii* leaves brought about by the increased expression of collagen 1 and fibronectin and the induction of migration of fibroblasts to the wound site. On comparing with my study, fibronectin plays an important role in wound healing and curcumin upregulates the gene expression and MMP-13, a multifunctional proteinase, in regulating multiple cellular functions including myofibroblast activity, cell motility, angiogenesis inflammation, and proteolysis during growth and maturation of wound granulation tissue also got upregulated on the effect of curcumin.

CONCLUSION

Skin provides a natural barrier against the environment and exerts a variety of essential protective functions. When the integrity of skin is compromised, either by acute or chronic injuries, the body initiates a multi-step and dynamic process at the injured site, leading to partial healing of the tissue and restoration of the skin's barrier function.

Turmeric (*Curcuma longa*), a rhizomatous perennial herb that has long been recognized for its medicinal use. Turmeric composed of different bioactive compounds including curcuminoids, turmerone, zingiberene, zedoaronediol, bisacurone etc. Curcumin (diferuloylmethane), is the major natural polyphenol found in the rhizome of turmeric has been shown different biological effects viz., anti-inflammatory, anti-oxidant, anti-carcinogenic and anti-infectious effects. In my study, the efficacy of 95% curcumin powder and supercritical extracted turmeric oil with more than 60% turmerones were determined using wound healing assay.

After conducting the wound healing assay, 6.25µg of Curcumin and 3.12µg of Turmeric oil were taken into consideration. Inorder to finalize the result, the gene expression of Fibronectin and MMP- 13 were conducted.

It has been clear from the Figure 6(a and b) that the concentration of curcumin has resulted in the up regulation of Fibronectin and the concentration of turmeric has resulted in down regulation when compared with Control. On considering MMP-13, curcumin leads to the up regulation of MMP-13 and turmeric leads to downregulation as compared to control.

In conclusion, it has been found that supercritically extracted curcumin possesses powerful modulating effects on wound healing than turmeric oil. Curcumin does this by acting on the inflammatory, proliferative and remodeling phases of the wound healing process and in doing so, reduces the time needed for wound healing.

REFERENCES

1. Adeliana, Usman, A. N., Ahmad, M., Arifuddin, S., Yulianty, R., & Prihantono. (2021). Effectiveness of turmeric (Curcuma longa Linn) gel extract (GE) on wound healing: Pre-clinical test. *Gaceta Sanitaria*, 35(S2):196–198. https://doi.org/10.1016/j.gaceta.2021.07.

2. Akbik, D., Ghadiri, M., Chrzanowski, W., & Rohanizadeh, R. (2014). Curcumin as a wound healing agent. *Life Sciences*, 116(1), 1–7. https://doi.org/10.1016/j.lfs.2014.08.016.

3. Barchitta, M., Maugeri, A., Favara, G., Magnano San Lio, R., Evola, G., Agodi, A., & Basile, G. (2019). Nutrition and wound healing: An overview focusing on the beneficial effects of curcumin. International Journal of Molecular Sciences, 20(5), 1119. https://doi.org/10.3390/ijms20051119

4. Borges, G. Á., Elias, S. T., da Silva, S. M., Magalhães, P. O., Macedo, S. B., Ribeiro, A. P., & Guerra, E. N. (2017). In vitro evaluation of wound healing and antimicrobial potential of ozone therapy. *Journal of Cranio-MaxillofacialSurgery*,45(3),364–370. https://doi.org/10.1016/j.jcms.2017.01.005

5. Bolla, S. R., Mohammed Al-Subaie, A., Yousuf Al-Jindan, R., Papayya Balakrishna, J., Kanchi Ravi, P., Veeraraghavan, V. P., Arumugam Pillai, A., Gollapalli, S. S., Palpath Joseph, J., & Surapaneni, K. M. (2019). In vitro wound healing potency of methanolic leaf extract of Aristolochia saccata is possibly mediated by its stimulatory effect on collagen-1 expression. *Heliyon*, 5(5). https://doi.org/10.1016/j.heliyon.2019.e01648

6. Chittasupho, C., Manthaisong, A., Okonogi, S., Tadtong, S., & Samee, W. (2021). Effects of quercetin and curcumin combination on antibacterial, antioxidant, in vitro wound healing and migration of human dermal fibroblast cells. *International Journal of Molecular Sciences*, 23(1), 142. https://doi.org/10.3390/ijms23010142

7. Chattopadhyay, Ishita ; Biswas, Kaushik ; Bandyopadhyay, Uday ; Banerjee,

Ranajit K (2004) Turmeric and curcumin: biological actions and medicinal applications. *Current science*, 87 (1):44-53.

8. Coaguila-Llerena, H., Rodrigues, E. M., Tanomaru-Filho, M., Guerreiro-Tanomaru, J. M., & Faria, G. (2019). Effects of calcium hypochlorite and octenidine hydrochloride on L929 and human periodontal ligament cells. *Brazilian Dental Journal*, 30(3), 213–219. https://doi.org/10.1590/0103-6440201902280

9. Dandannavar, V., Balakrishna, J., Dhawale, L., Joseph, J., Gollapalli, S., Pillai, A., & Veeraraghavan, V. (2019). Wound-healing potential of methanolic extract of Rhaphidophora korthalsii leaves possibly mediated by collagen and fibronectin expression in L929 cell line. National Journal of Physiology, Pharmacy and Pharmacology, 1. https://doi.org/10.5455/njppp.2019.9.0623404072019

10. David Cadagan and Stephen Merry. (2013). Circumvention of Inherent or Acquired Cytotoxic Drug Resistance . *In Vitro* Using Combinations of Modulating Agents. *International Journal of Cancer Research and Treatment* ; 33(10):4381-4387.

11. Emiroglu, G., Ozergin Coskun, Z., Kalkan, Y., Celebi Erdivanli, O., Tumkaya, L., Terzi, S., Özgür, A., Demirci, M., & Dursun, E. (2017). The effects of curcumin on wound healing in a rat model of nasal mucosal trauma. *Evidence-Based Complementary and Alternative Medicine*, 2017, 1–6. https://doi.org/10.1155/2017/9452392.

12. Freshney, R. I. (2005). Cryopreservation. Culture of Animal Cells. https://doi.org/10.1002/0471747599.cac020.

13. Fronza, M., Heinzmann, B., Hamburger, M., Laufer, S., & Merfort, I. (2009). Determination of the wound healing effect of calendula extracts using the scratch assay with 3T3 fibroblasts. *Journal of Ethnopharmacology*, 126(3), 463–467. https://doi.org/10.1016/j.jep.2009.09.014

14. Hosseinkhani, A., Falahatzadeh, M., Raoofi, E., & Zarshenas, M. M. (2016). An evidence-based review on wound healing herbal remedies from reports of traditional Persian medicine. *Journal of Evidence-Based*

Complementary & Alternative Medicine, 22(2), 334–343. https://doi.org/10.1177/2156587216654773.

15. Kaptaner İğci, B., & Aytaç, Z. (2020). An investigation on the in vitro wound healing activity and phytochemical composition of Hypericum pseudolaeve Robson growing in Turkey. *Turkish Journal of Pharmaceutical Sciences*; 17(6):610-619. https://doi.org/10.4274/tjps.

16. Li, B., & Wang, J. H.-C. (2011). Fibroblasts and myofibroblasts in wound healing: Force generation and measurement. *Journal of Tissue Viability*, 20(4), 108–120. https://doi.org/10.1016/j.jtv.2009.11.004

17. Maver, T., Maver, U., Stana Kleinschek, K., Smrke, D. M., & Kreft, S. (2015). A review of herbal medicines in wound healing. International Journal of Dermatology, 54(7), 740–751. https://doi.org/10.1111/ijd.12766.

18. Mathew-Steiner, S. S., Roy, S., & Sen, C. K. (2021). Collagen in wound healing.*Bioengineering*,8(5),63. https://doi.org/10.3390/bioengineering8050063

19. P., B. (2013). Wound healing and the role of fibroblasts. *Journal of Wound Care*, 22(8), 407–412. https://doi.org/10.12968/jowc.2013.22.8.407

20. Prasad, S., & amp; Aggarwal, B. (2011). Turmeric, the golden spice. *Oxidative Stress and Disease*, 263–288. https://doi.org/10.1201/b10787-14

21. Reddy, K.J., Bahadur, B., Bhadraiah, B., & Rao, M.L.N. (2005). Medicinal Plants: An Overview. *Advances in Medicinal Plants*; 2005: 1-52.

22. Rodriguez, L. G., Wu, X., & Guan, J.-L. (n.d.). Wound-healing assay. Cell Migration, *Methods of Molecular Biology Series*; 294: 023–030. https://doi.org/10.1385/1-59259-860-9:023.

23. Sharifi-Rad, J., Rayess, Y. E., Rizk, A. A., Sadaka, C., Zgheib, R., Zam, W., Sestito, S., Rapposelli, S., Neffe-Skocińska, K., Zielińska, D., Salehi, B., Setzer, W. N., Dosoky, N. S., Taheri, Y., El Beyrouthy, M., Martorell, M., Ostrander, E. A., Suleria, H. A., Cho, W. C., … Martins, N. (2020). Turmeric

and its major compound curcumin on health: Bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Frontiers in Pharmacology*, 11. https://doi.org/10.3389/fphar.2020.01021.

24. Sharma, A., Khanna, S., Kaur, G., & Singh, I. (2021). Medicinal plants and their components for wound healing applications. *Future Journal of Pharmaceutical Sciences*, 7(1):53. doi:10.1186/s43094-021-00202-w

25. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines*, 10(5). https://doi.org/10.4314/ajtcam.v10i5.2.

26. Tejada, S., Manayi, A., Daglia, M., F. Nabavi, S., Sureda, A., Hajheydari, Z., Gortzi, O., Pazoki-Toroudi, H., & M. Nabavi, S. (2016). Wound healing effects of Curcumin: A short review. *Current Pharmaceutical Biotechnology*, 17(11), 1002–1007. https://doi.org/10.2174/1389201017666160721123109

27. Toden, S., Theiss, A. L., Wang, X., & Goel, A. (2017). Essential turmeric oils enhance anti-inflammatory efficacy of curcumin in dextran sulfate sodium-induced colitis. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-00812-6

28. Udayakumar, G. P. (2020). Evaluation of wound healing capacity of selected leaf extracts using in vitro scratch assay with L929 fibroblasts. *Bioscience Biotechnology Research Communications,* 13(11), 66–69. https://doi.org/10.21786/bbrc/13.11/15

29. Velnar, T., Bailey, T., & Smrkolj, V. (2009). The wound healing process: An overview of the cellular and molecular mechanisms. *Journal of International Medical Research*, 37(5), 1528–1542. https://doi.org/10.1177/147323000903700531.

30. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. Molecules, 21(5), 559.

https://doi.org/10.3390/molecules21050559.