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ORIGINAL RESEARCH ARTICLE

Bioscreening and pre-clinical evaluation of the impact of bioactive molecules from *Ptychotis verticillata* on the multilineage potential of mesenchymal stromal cells towards immune- and inflammation-mediated diseases

Fatima Bouhtit[1,2] · Mehdi Najar[3,4,9] · Saida Rahmani[1,2] · Rahma Melki[2] · Mustapha Najimi[5] · Khalid Sadki[6] · Noreddine Boukhatem[2] · Jean-Claude Twizere[7,8] · Nathalie Meuleman[1] · Philippe Lewalle[1] · Laurence Lagneaux[3] · Makram Merimi[1,2]

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Abstract

Objective and design Mesenchymal stromal cells (MSCs) are currently used in cell reparative medicine due to their trophic and ant-inflammatory properties. The modulation of stem cell properties by phytochemicals has been suggested as a tool to empower their tissue repair capacity. In vitro, MSCs are characterized by their tri-lineage potential that holds great interest for tissue regeneration. *Ptychotis Verticillata* (PV), an aromatic and medicinal plant, may be thus used to modulate the in vitro multilineage potential of MSCs.

Materials and methods We screened the impact of PV-derived essential oil and their bioactive molecules (thymol and carvacrol) on the in vitro multilineage potential of MSCs. Different concentrations and incubation times of these compounds were assessed during the osteogenesis and adipogenesis of MSCs.

Results The analysis of 75 conditions indicates that these compounds are biologically active by promoting two major differentiation lineages from MSCs. In a time- and dose-dependent manner, thymol and carvacrol increased the osteogenesis and adipogenesis.

Conclusion According to these preliminary observations, the addition of PV extract may stimulate the tissue regenerative and repair functions of MSCs. Further optimization of compound extraction and characterization from PV as well as cell treatment conditions should increase their therapeutic value in combination with MSCs.

Keywords MSCs · Multilineage potential · Medicinal plants · Thymol and carvacrol · Essential oil · Reparative medicine

Introduction

The increase of diseases prevalence related to tissue injury is a leading cause of morbidity and disability [1]. Regenerative medicine is an emerging field that seeks to repair injured tissues by using stem cells. Immune-mediated mechanisms of regeneration and repair may promote the functional regrowth of vital tissues. Consequently, the major limiting step for such strategy is to find and use therapeutic stem/progenitor cells with a well-defined and enhanced therapeutic efficiency [2, 3]. Mesenchymal stromal cells (MSCs) are increasingly indicated for tissue repair applications. Virtually present in almost all tissues, MSCs are considered multipotent progenitors with the capacity to differentiate into connective tissue cells. MSCs were originally isolated from bone marrow (BM) which represents actually the main source used for both in vitro and in vivo studies. Among the minimal criteria to define MSCs, as established by the International Society for Cellular Therapy, there is their tri-lineage differentiation potential. Under appropriate culture conditions, MSCs can differentiate into adipocytes, chondrocytes, and osteocytes

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Fatima Bouhtit and Mehdi Najar contributed equally to this study.

Laurence Lagneaux and Makram Merimi are equal senior authors.

✉ Mehdi Najar
mnajar@ulb.ac.be

Extended author information available on the last page of the article

[4]. This in vitro differentiation assay allows investigating the possible regenerative properties of MSCs and therefore their capacity to repair injured tissue by local cellular differentiation [5]. A series of experiments have revealed that replacement of damaged or defective cells by exogenously administered stem cells is critical in achieving their therapeutic effects in various diseases [6]. Upon arriving in damaged tissue, MSCs are believed to exert their therapeutic effects in two ways: by cell replacement and by cell 'empowerment'. Many studies have attempted to exploit the potential of MSCs to differentiate and thus replace damaged resident cells [7].

Despite that they are extensively used for maintaining the differentiation profile of stem cells, biological factors such as recombinant and synthetic cytokines, growth factors, and other proteins have several issues that hamper their widespread use in cell-therapy. The advantage of using MSCs over a recombinant growth factor cocktail-based therapy relies on the unique capacity of MSCs to sense and re-establish a reparative and regenerative local environment in response to changing needs (damage, inflammation, infection, oxidative stress) of the wound [8]. MSCs are sensitive to their surroundings and may utilize rheostatic (sensing) mechanisms to respond by adapting their fate and behavior [9, 10]. This adaptive response to cues from the microenvironment depends on distinct intracellular signaling, changes of the transcriptome, and metabolic reprogramming [11]. Their metabolic properties in terms of sensing, reacting, and producing metabolites influencing tissue inflammation are essential to achieve tissue regeneration and rejuvenation [12].

Plant is still a key reservoir of molecules with medicinal attributes. Recent advances in the field of herbal medicine coupled with achievement in stem cell therapy have proposed new therapeutic strategies for tissue regeneration [13]. Indeed, natural bioactive compounds may modulate the self-renewal and differentiation potential of adult stem cells [14]. The importance of phytochemicals-based modulation of stem cell fate has been discussed [15]. Osteoinductive coumpounds including silibinin, resveratrol, quercetin and genistein were thus shown to promote the osteogenic differentiation of BM-MSCs. In parallel, adipoinductive coumpound such as genistein was reported to stimulate the adipogenic differentiation of BM-MSCs. As a new potential source of bioactive molecules, *Ptychotis verticillata* (PV) from Morocco is increasingly considered and used as a therapeutic medicinal plant [2, 15]. However, a review of the literature from PubMed indicated that only two studies have explored the potential effects of this plant. The characterization of essential oil is made difficult by their complexity and by the different compositions present in the same oil having different geographical origins [16]. Classified as phytochemicals, thymol and carvacrol are the major compounds of PV. As shown in Table 1, thymol and carvacrol are the main compounds of PV, with specific structures and chemical properties. They display several biological effects including stimulation of cell growth and protection against oxidative stress [3]. Accordingly, promoting the multilineage potential of MSCs by thymol and carvacrol may sustain the tissue repair process. These compounds may stimulate the osteogenesis and adipogenesis in a time- and dose-dependent manner. These preliminary observations pave the way for the use of PV extract in combination with MSCs. Further screening should improve our understanding of these compounds as well as of their effects to guarantee an efficient therapeutic strategy towards immune- and inflammation-mediated diseases.

Materials and methods

Isolation, culture, expansion, and characterization of MSCs

The samples [4] were collected after approval by the local ethics committee of Institut Jules Bordet and according to the recognized guidelines of the Helsinki Declaration. Informed written consent was obtained for all donors. BM was harvested from the sternum or iliac crest of five healthy volunteers. The mean age of the donors was 33 ± 2 years (18–41 years). Mononuclear cells (MNCs) were isolated from bone marrow aspirates by density-gradient centrifugation (Linfosep; Biomedics) and washed in the Hank's buffered salt solution (HBSS; Lonza). MNCs were seeded at a cell density of 2×10^4 cells/cm^2 in a low-glucose DMEM (DMEM-LG; Lonza) supplemented with 10% (v/v) heat-inactivated FBS, 2mML glutamine, and 0.5% (v/v) antibiotic/antimycotic solution (all from Life Technologies). Cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. After 48 h, non-adherent cells were removed by washing and the medium (DMEM-LG) was changed twice a week. When sub-confluency (80–90%) was achieved, adherent cells were recovered by adding TryplSelect solution (Lonza) and expanded by replating at a lower density (200 cells/cm^2) using DMEM-LG as culture medium. The immunophenotype of MSCs was established by flow cytometry using the monoclonal antibodies as previously indicated [17]. The data were acquired and analyzed on a MacsQuant analyzer (Miltenyi Biotec, Leiden, The Netherlands).

Plant material

The aerial parts of PV were collected during the flowering season in May 2018 and 2019 (full bloom) from Morocco. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco. Fresh vegetal material was water distillated (3 h) using a Clevenger-type apparatus according to the method recommended in the

Table 1 Structure, identification and properties of thymol and carvacrol

	Thymol	Carvacrol
Structure		
Identification		
Synonym(s)	5-Methyl-2-isopropylphenol, 2-Isopropyl-5-methylphenol, 5-Methyl-2-(1-methylethyl) phenol, IPMP	5-Isopropyl-2-methylphenol
Color	Colorless to white	Colorless to yellow to orange
Form	Crystalline	Liquid
Chemical properties		
Linear formula	2[(CH3)2CH]C6H3-5-(CH3) OH	(CH3)2CHC6H3(CH3) OH
Molecular weight (g/mol)	150.22	150.22
Physical properties		
Solubility	Ethanol	Ethanol, diethyl ether, carbon tetrachloride, acetone
Density (g/ml)	0.969	0.976
Purity (HPLC) (%)	> 98.5	≥ 97.5
T° fusion (°C)	49.6	2.5
T° boiling (°C)	233	236.85
Volumic mass ($g \cdot cm^{-3}$)	0.97–0.93	0.9772–0.98

European Pharmacopoeia (Council of Europe, 1996). The essential oil yields were 2% (w/w). The oils were dried over anhydrous sodium sulfate and then stored in sealed glass vials at ambient temperature prior to analysis.

Chemical reagents

All chemicals, unless otherwise stated, were of the highest quality and were used as supplied. Carvacrol (99.9%) and thymol (98.5%) were purchased from Sigma-Aldrich (St. Louis, USA).

Preparation of essential oil, carvacrol and thymol solutions

Essential oil

Fifty µl of pure essential oil was dissolved in 50 µl dimethylsulfoxide (DMSO, Merck, Germany) and diluted (0.01%, 0.025%) with culture medium before experiments.

Carvacrol

One hundred µl of pure Carvacrol was dissolved in 900 µl DMSO and diluted (6 µM and 25 µM) with culture medium before experiments.

Thymol

One hundred mg of thymol powder was dissolved in 1 ml DMSO and diluted (3 µg/ml and 6 µg/ml) with culture medium before experiments.

Addition to culture of MSCs

Different solutions of essential oil, carvacrol and thymol were added to BM-MSC culture for 24 h and 72 h of incubation.

Multilineage potential of MSCs

For the in vitro multilineage potential of MSCs in the presence or not of *Ptychotis verticillata* compounds extract, we have presented the analysis of 75 representative conditions taking into account the impact of time incubation and dose concentration effects.

Osteogenic differentiation

Five thousand cells/well were plated in a 24-well plate with culture medium. After 5 days, the medium was completely discarded and replaced by osteogenic medium

(StemMACS OsteoDiff Media, Miltenyi Biotec). Cells were fed weekly with fresh osteogenic medium. After 7, 14 and 21 days, the mineralization of the extracellular matrix was assessed by Alizarin Red staining. Cells were washed in phosphate-buffered saline (PBS) and fixed in 70% ethanol at room temperature for 5 min followed by several washes in H_2O. Cells were stained in 40 mM Alizarin red (Sigma-Aldrich) pH 4.2 for 15 min at room temperature, rinsed in H_2O, and then air dried. The red staining was examined by light microscopy.

Adipogenic differentiation

5,000 cells/well were plated in a 24-well plate with culture medium. After 5 days, the medium was completely discarded and replaced with adipogenic medium (StemMACS AdipoDiff Media, Miltenyi Biotec). Cells were fed weekly with fresh adipogenic medium. At day 7, cells were stained with Oil Red O solution (Sigma) after fixing (8% formaldehyde). Lipid vacuoles were then observed by light microscopy.

Controls

The negative controls are MSCs cultured in classical medium. The positive controls are MSCs cultured in specific induction medium for both osteogenic and adipogenic differentiation. Moreover, cortisol is also used as positive control to induce adipogenic differentiation.

Results and discussion

Mesenchymal stromal cells (MSCs) represent a progenitor cell population with several therapeutic properties. Initially isolated from bone marrow, MSCs can be derived from a variety of tissues [10]. MSCs have drawn much attention during the last decade in the field of regenerative medicine. As common progenitor cells of adipocytes and osteoblasts, MSCs are delicately balanced for their differentiation commitment. A variety of external cues contribute to the delicate balance of adipo-osteogenic differentiation of MSCs [18]. Their healing functions may rely on their capacity to differentiate into functional specific local cell types, mobilization and stimulation of other local progenitors, the production of diverse elements of the extracellular matrix, growth factors, cytoprotective mediators, and cytokines [19, 20]. The long-term survival and/or engraftment of differentiated MSCs within the injured tissue is linked to their surroundings. Indeed, MSCs are sensitive and may differentially respond to distinct challenges. The choice of source of isolation, culture medium, and conditions may also affect some features of MSCs [19]. Thus, several strategies including drug-based combination approaches have been proposed to enhance the efficiency of MSCs [9, 20, 21]. Although their mechanisms of action remain undetermined, medicinal plants represent a drugstore with several bioactive molecules. In line, plant-derived compounds can be combined with stem cells as a new promising perspective to promote tissue regeneration and repair. Compared to cytokines and growth factors [22], plant-derived products are likely more safer and efficient as differentiation factors for stem cells [23]. Due to their plasticity, MSCs might be modulated by natural compounds to strengthen their therapeutic value during tissue repair [13]. In this study, we investigated the effects of thymol, carvacrol and the essential oil from PV on the multilineage potential of MSCs [3, 15]. Due to their simple and easy isolation procedure as well as their great expansion potential, MSCs are ideal candidates for regenerative medicine [14]. MSCs are highly clonogenic stromal progenitor cells with a specific profile and role [24, 25]. Once obtained, MSCs were able to adhere to plastic and responded to the ISCT criteria. They displayed a fibroblastic-like shape and showed positive (> 95%) expression for CD73, CD90, and CD105 and negative (< 5%) expression for CD14, CD19, CD34, CD45, and HLA-DR markers (Fig. 1).

The analysis of the chemical composition of *Ptychotis verticillata* essential oil from Morocco was carried out using gas chromatography–mass spectrometry (GC–MS) technology [15]. The EO was dominated by phenolic compounds (48.0%) with carvacrol (44.6%) and thymol (3.4%) as the main compounds. Second, carvacrol and thymol being quite structurally similar, display distinct biological properties. There are other components present in the extract of thymol and carvacrol. In fact, the chemical composition of PV EO from Morocco revealed the presence of 19 constituents, which accounted for 98.9% of the total oil [15]. These compounds include thujene, pinene, sabinene, pinene, myrcene, cymene, limonene, cineole-1,8, terpinene, linalool, borneol, terpinen-4-ol, terpineol, carvacryl methyl ether, thymol, carvacrol, terpinyl acetate, geranyl acetate, and caryophyllene oxide. The choice to study combination is linked to several reasons. The metabolism of carvacrol and thymol in rats was studied using gas chromatographic–mass spectrometric methods. The urinary excretion of metabolites was rapid. Only very small amounts were excreted after 24 h. Although large quantities of carvacrol and, especially, thymol were excreted unchanged (or as their glucuronide and sulfate conjugates), extensive oxidation of the methyl and isopropyl groups also occurred [26]. The plasma pharmacokinetics of thymol and carvacrol was also previously estimated [27]. The plasma half-lives were short for thymol (approximately, 1.6 h) and carvacrol (approximately, 1.5 h), whereas the estimated half-lives for these substances in tissues ranged from 13.9 to 31.5 h for thymol and from 16.9 to 25 h for carvacrol. The predicted amount of time that the molecules would be

found in the body based on the slowest depletion time of liver tissue was 13 days for thymol and 10 days for carvacrol. The apparent half-life of topically applied carvacrol was approximately 4.5 h in plasma, with an estimated withhold time of 10 days.

As carvacrol and thymol are the two main active phenolic compounds found in *Ptychotis verticillata* (PV) and other medicinal plants, we decided to assess their capacity to enhance the multilineage potential of MSCs by in vitro assays. The analysis of 75 conditions indicate that these compounds are biologically active by promoting two major differentiation lineages from MSCs. Compared to controls, we found that both the osteogenic (Fig. 2) and adipogenic (Fig. 3) differentiation potential of MSCs were induced. Regardless of their type, all the compounds have increased these two differentiation lineages from MSCs in a time- and dose-dependent manner. Indeed, from day 7 to day 21, there is a consistent and substantial increase in the level of differentiation in the presence of high concentrations of the compounds. The mineralization of the extracellular matrix as stained by the Alizarin Red solution was significantly enhanced, confirming the stimulating effects of PV extract on the osteogenesis of MSCs. In parallel, the accumulation of lipidic vacuoles as stained with Oil Red O solution was also substantially increased confirming the boosting effects of PV extract on the adipogenesis of MSCs. Accordingly, such compounds have demonstrated positive effects on MSC multilineage and may thus promote the tissue repair functions of MSCs. Stimulating bone growth and regeneration, especially in patients with delayed union or non-union of bone, is a challenge for orthopedic surgeons [28]. MSCs play a key role in fracture repair by differentiating to become bone-forming osteoblasts. MSCs can be therapeutically manipulated to promote bone differentiation and healing [29]. Pharmacologically manipulating the number and differentiation capacity of MSCs is one potential therapeutic approach to improve healing. In agreement, plant-derived biomaterials have been reported to possess osteoconductive properties that contribute to bone formation and repair [23]. Osteoinduction effects such as that delivered by PV-derived compounds may be considered as a signal to progenitor cells to differentiate toward the required cell-tissue lineage. Indeed, stromal cells which are located in specific niches maintain the tissue homeostasis by regulating the quantity of primitive and committed cells within the tissue [13]. Signals from the environment regulate and control

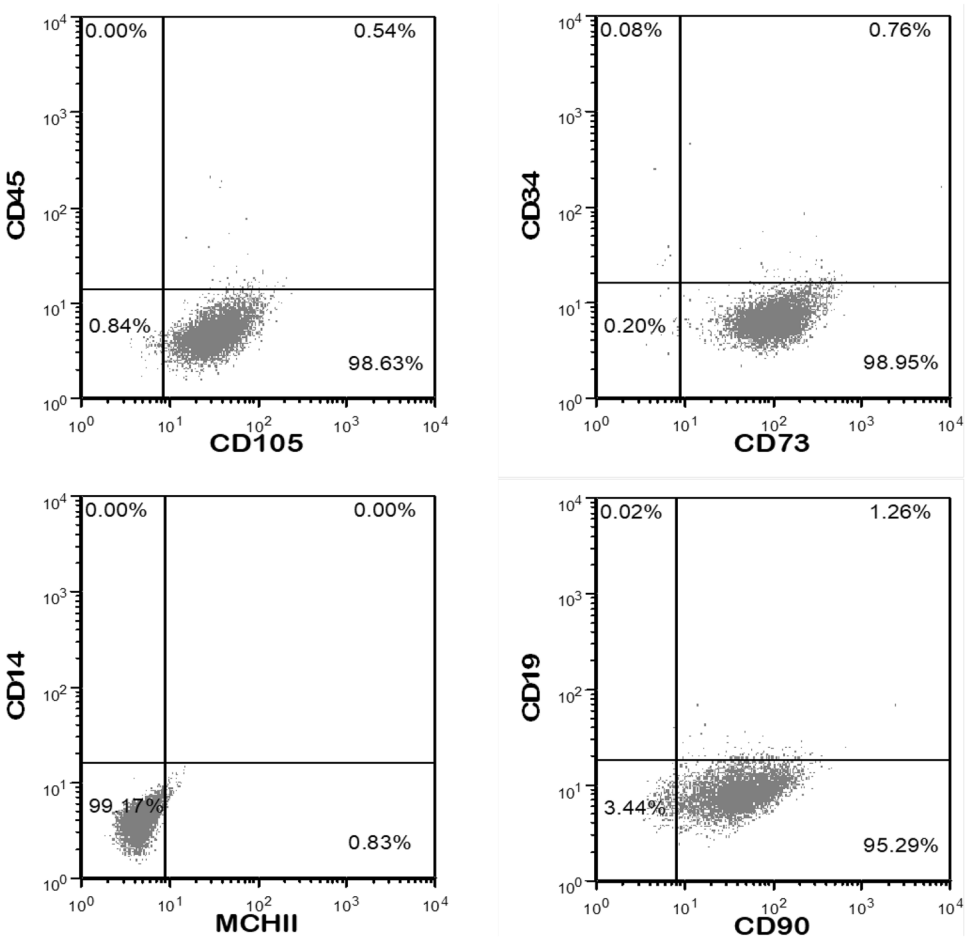

Fig. 1 Immunophenotype of MSCs. MSCs stained using specific fluorochrome-labeled monoclonal antibodies against CD14, CD19, CD34, CD45, HLA-DR, CD73, CD90, and CD105

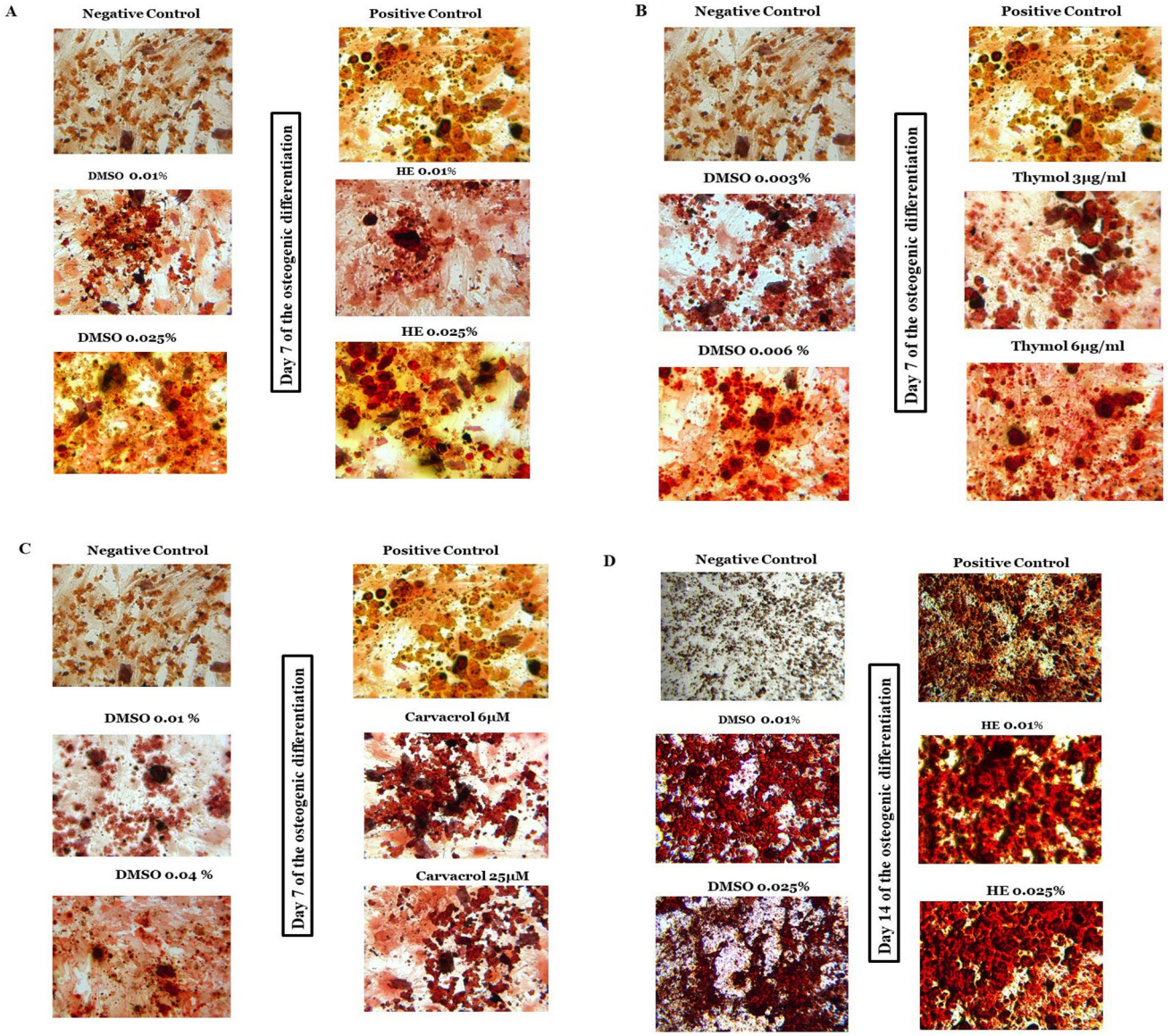

Fig. 2 Osteogenic differentiation potential of MSCs. MSCs were cultivated, under the indicated time (7, 14 and 21 days), alone or in the presence of different concentrations of essential oil, thymol and carvacrol. The mineralization of the extracellular matrix was stained by the Alizarin Red solution. **A**, **B** and **C** correspond to the treatment of MSCs with essential oil, thymol and carvacrol, respectively, during 7 days of the osteogenic differentiation. **D**, **E** and **F** correspond to treatment of MSCs with essential oil, thymol and carvacrol, respectively, during 14 days of osteogenic differentiation. **G**, **H** and **I** correspond to the treatment of MSCs with essential oil, thymol and carvacrol, respectively, during 21 days of osteogenic differentiation

the balance of self-renewal and differentiation capacity of stem/progenitor cells residing in the tissue. To facilitate the generation of precursor cells with specific differentiated functions, various growth factors, cytokines, hormones, and other biological molecules could be used [14]. The multilineage assay demonstrated that MSCs can generate adipocytes and osteoblasts. MSCs are able to replicate and differentiate to different lineages [30] depending on the tissue needs. In fact, MSCs are reported to be promoters, enhancers, and playmakers of translational regenerative medicine [31]. As demonstrated by this study, the use of PV extracts is able to boost the multilineage of MSCs and therefore their tissue repair functions. Indeed, during tissue injury, MSCs are recruited to the site of inflammation and differentiate into two important precursors, adipocytes, or osteoblasts, leading to bone repair and regeneration. With this increased multilineage potentiality, MSCs may be used to cure wounding. Fructus Ligustri Lucidi (FLL), which is used in traditional Chinese medicine, was demonstrated to be capable of enhancing osteogenic differentiation of MSCs

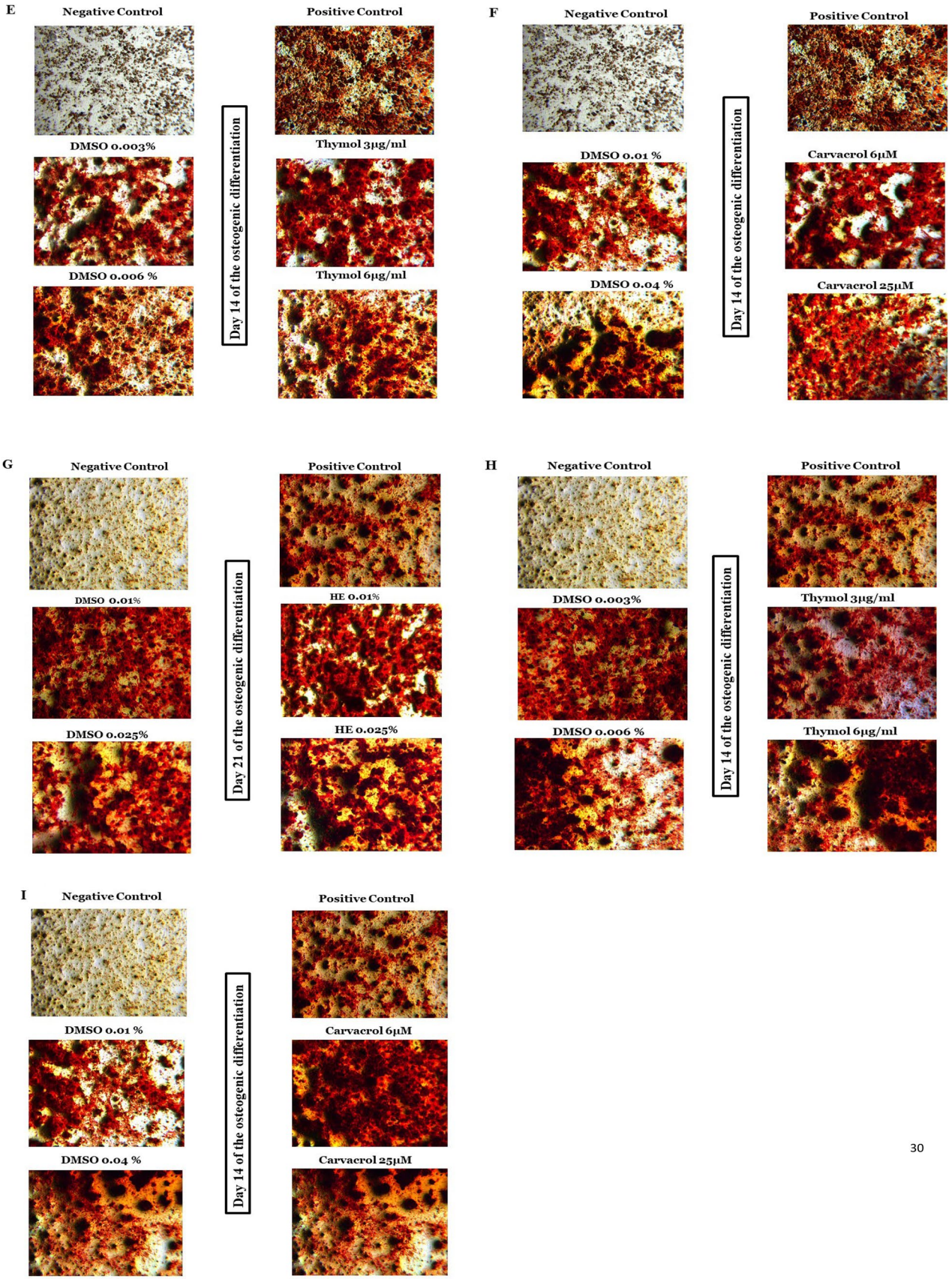

Fig. 2 (continued)

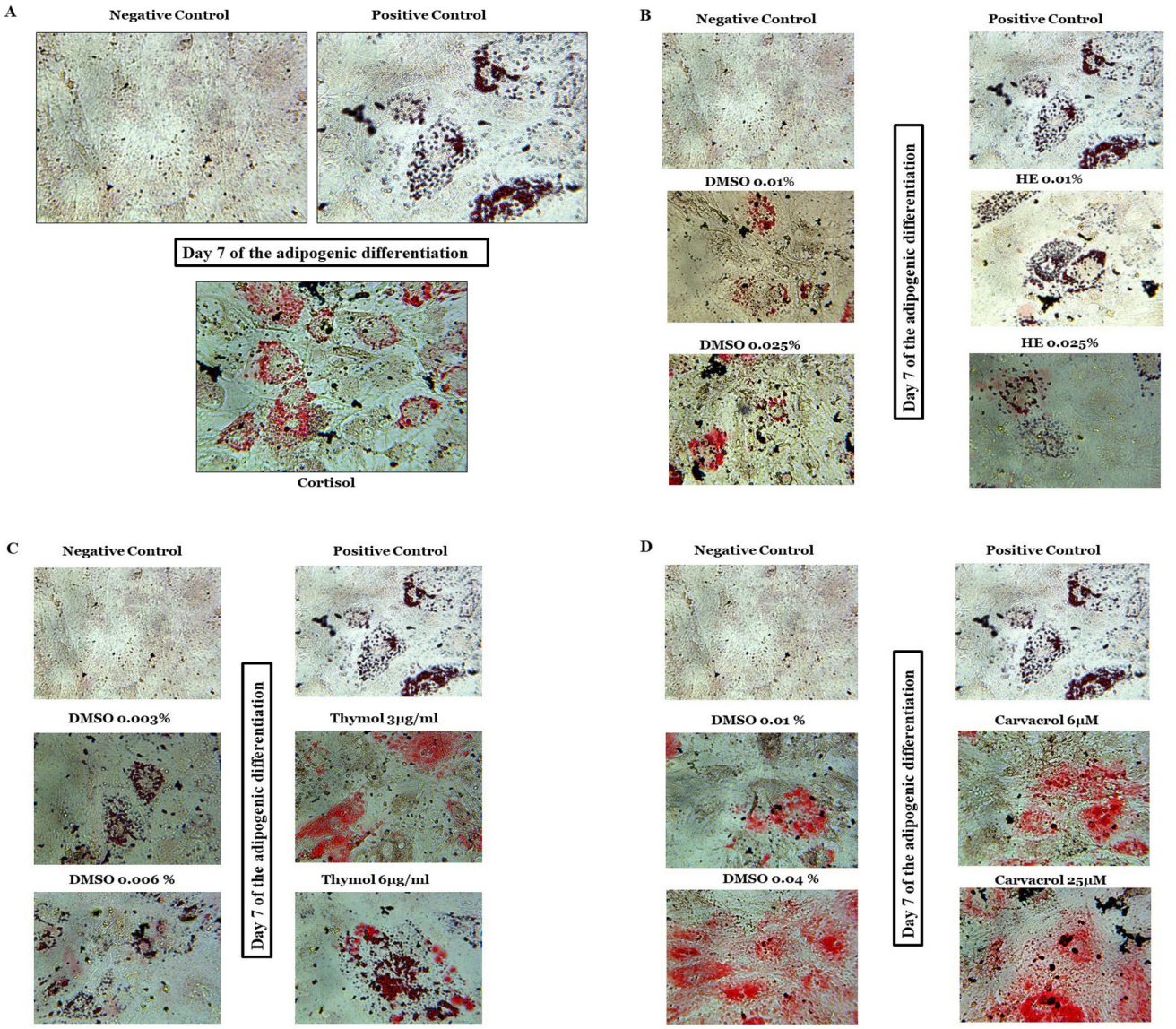

Fig. 3 Adipogenic differentiation potential of MSCs. MSCs were cultivated under the indicated time (7 days), alone or in the presence of different concentrations of essential oil, thymol and carvacrol. The accumulation of the lipidic vacuoles was stained with Oil Red O solution. **A**, **B**, **C** and **D** correspond to treatment of MSCs with controls, essential oil, thymol and carvacrol, respectively, during 7 days of adipogenic differentiation

[32]. Further studies indicated that pre-differentiated osteogenetic MSCs-supplemented scaffold had superior healing effects [33]. During tissue healing, undifferentiated MSCs proliferate and differentiate, thereby repairing the injury. Providing MSCs with enhanced differentiation rate may help in ensuring high therapeutic effects. Strategies to enhance tissue healing are being investigated with variations in the model, sources of stem cells, and methods for promoting stem cell activity [34]. Bone defect is an important topic in public health. Novel therapies are based on osteogenic induction by natural anti-osteoporotic compounds including plant-derived estrogens. The osteogenic induction potential of *Sophora pachycarpa* root extract (SPRE) was reported on human adipose-derived MSCs. In the presence of increased concentrations of the extract and during 21 days, the osteogenesis capacity of MSCs was greatly induced [35]. Because their engraftment is generally considered low and transient, priming by such bioactive molecules would be of benefit to increase MSC survival and functionality when transplanted in vivo. Indeed, the enrichment of grafted tissue with ADSCs harboring enhanced adipogenic and osteogenic differentiation potential allows increasing the survival and

retention of the grafts [36]. In line, thymol/carvacrol and the essential oil have been shown to improve wound healing by stimulating tissue development as well as promoting survival and growth of fibroblasts and keratinocytes [37].

Thymol, chemically known as 2-isopropyl-5-methylphenol, is a colorless crystalline monoterpene phenol. It is one of the most important dietary constituents in thyme species [38]. Thymol extracted from Thymus vulgaris (thyme) increased cell adhesion, proliferation and osteogenic potential of dental pulp MSCs. A significant production of calcium in the matrix, following functionalization with bone morphogenetic protein-2 (BMPs), was evidenced [39]. Multiple BMPs, including BMP2, BMP6, BMP7 and BMP9, promote osteoblastic differentiation of MSCs both in vitro and in vivo [40]. Supplementing the culture medium of human adipose tissue-derived MSCs with oil rich in thymol of *Lippia origanoides* increased cell proliferation, especially at later passages [41]. This essential oil has the potential to become a cheap and efficient means for optimally expanding adult stem cells in vitro for cell therapy. Thymol enhanced expression of a core set of brown fat-specific markers as well as increased protein levels of PPARγ, PPARδ, pAMPK, pACC, HSL, PLIN, CPT1, ACO, PGC-1α, and UCP1, suggesting its possible role in promoting adipocytes [42].

Carvacrol, chemically known as 2-methyl-5-(1-methylethyl)-phenol, is a phenolic monoterpenoid and isomer with thymol. Carvacrol exhibits broad-spectrum bioactivity that may be useful in pharmacology, as an antimicrobial, antioxidant and anticancer agent [43]. Carvacrol has been shown to promote the angiogenesis and endothelial differentiation of MSCs which may participate in tissue regeneration [44]. Carvacrol as a component of thyme oil was identified as an activator of the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR) pathway [45], which may enhance and accelerate the adipogenic differentiation of MSCs in vitro and in vivo [46]. PPAR-ɣ has been proposed to be a major decision factor in MSC lineage commitment by promoting adipogenesis from MSCs [47]. In parallel, *Solanum muricatum* extract promoted the osteogenic differentiation of MSCs, ameliorating thus the symptoms of osteogenesis imperfecta in a rat vivo model [48]. The combined administration of *Ptychotis verticillata* extract and MSCs may represent a new approach to enhance the therapeutic issue. Indeed, essential oil, thymol and carvacrol from *Ptychotis verticillata* have shown positive effects on MSCs by preserving their morphology, sustaining their viability, promoting their proliferation, protecting them from cytotoxicity and oxidative stress [49]. The viability of MSCs is enhanced in a time- and dose-dependent manner that may be associated with improving the therapeutic efficacy. Herein, we have reported that these compounds, in a time- and dose-dependent manner, may increase the in vitro osteogenesis and adipogenesis of MSCs. Accordingly, the addition of PV extract may stimulate the tissue regenerative and repair functions of MSCs.

Conclusion

MSCs participate in homeostasis by repairing tissue, inhibiting inflammation, and regulating immune responses. As adjuvant strategies, phytochemicals are emphasized to strengthen the potential of MSCs. The derived constituents are biologically active by showing positive effects on the multilineage properties of MSCs in vitro. Carvacrol and thymol significantly sustained in vitro the osteogenic and adipogenic capacities of MSCs. Such effect may represent a new approach to increase the trophic value of MSCs. Thus, PV holds great potential for developing new drugs to improve the healing of injured tissue by MSCs. These preliminary in vitro results are beneficial for the field of regenerative medicine and deserve more screening in follow-up studies. However, some limitations have to be reported to increase the relevance of the results. Due to their limited number, after isolation, in vitro culture and expansion of MSCs are required to achieve a sufficient number of cells for clinical and research applications. In addition, increasing the size number of the sample will strengthen the significance of the results. The in vitro results might be different from those obtained from the in vivo condition. Thus, animal models should be developed to properly reflect what is happening in vivo. Such results are encouraging and have to be confirmed by both gene and protein observations. Finally, the amelioration of compound manufacturing, characterization and controlled release will increase their biological and therapeutic value. Further in vivo and clinical studies are necessary to understand their bioavailability, pharmacokinetics and mechanism of actions. Unfortunately, the low cost that these EOs have on average and their non-patentability make them little or not at all interesting for pharmaceutical industries, making missing the sponsors for the clinical studies necessary to finally validate their therapeutic efficacy in many different pathologies.

Perspectives

Determining the phenotype and immunomodulatory profile of MSCs after treatment by these compounds are highly recommended to ensure their functional immunotherapeutic impact. Carvacrol and thymol could contribute to the reduction of inflammatory responses through modulation of the expression of JNK, STAT-3, AP-1, and NFATs [50]. Carvacrol essential oils may suppress the immune response and inflammation-related gene expression in broilers challenged by lipopolysaccharide (LPS). Orally administered

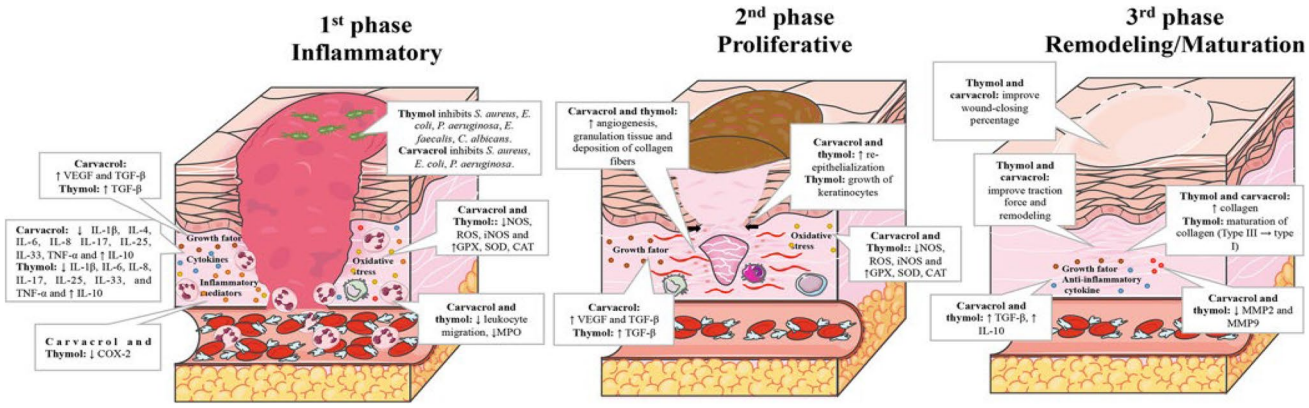

Fig. 4 Perspectives associated with carvacrol, thymol and essential oils on wound healing. Effects of carvacrol, thymol and essential oils containing such monoterpenes on wound healing: a systematic review. Reprinted from Journal of Pharmacy and Pharmacology, Volume: 71, Issue: 2, Pages: 141–155. Michelle Fonseca Costa, Aimée Obolari Durço, Thallita Kelly Rabelo, Rosana de Souza Siqueira Barreto, Adriana Gibara Guimarães. Effects of carvacrol, thymol and essential oils containing such monoterpenes on wound healing: a systematic review. PMID: 30537169 https://doi.org/10.1111/jphp.13054. This figure has been reproduced with permission from PHARMACEUTICA L PRESS under License ID 1146258–1 and ISSN 0022-3573

carvacrol essential oils inhibited the secretion of inflammatory cytokines caused by LPS, affected the pathway of TLRs/NF-κB, and showed an anti-inflammatory function [51]. Moreover, several studies suggest that these compounds are strong candidates for the development of new wound treatment products [37]. Due to their vast pharmacological and biological action, carvacrol and thymol or essential oils containing at least one of these compounds are strong candidates for the development of future drugs for the management of tissue repair. Thymol/carvacrol was able to act in the three phases of wound healing as shown in Fig. 4. In the first phase, they showed modulatory effect of the inflammatory cytokines, oxidative stress and antimicrobial power. In the second phase, they promoted re-epithelialization, angiogenesis and development of granulation tissue. Finally, in the third phase, they improve the collagen deposition and modulated the growth of fibroblasts and keratinocytes [37]. The determination of the doses and formulation and the effectiveness and safety of the use of these compounds or essential oil containing them for the treatment of wounds, as well as the mechanisms by which these effects can be observed, stand out as the challenges for future studies.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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Authors and Affiliations

Fatima Bouhtit[1,2] · Mehdi Najar[3,4,9] · Saida Rahmani[1,2] · Rahma Melki[2] · Mustapha Najimi[5] · Khalid Sadki[6] · Noreddine Boukhatem[2] · Jean-Claude Twizere[7,8] · Nathalie Meuleman[1] · Philippe Lewalle[1] · Laurence Lagneaux[3] · Makram Merimi[1,2]

Fatima Bouhtit
bouhtitfatima@gmail.com

Saida Rahmani
saida.ramani@gmail.com

Rahma Melki
r.melki@ump.ac.ma

Mustapha Najimi
mustapha.najimi@uclouvain.be

Khalid Sadki
ksadki1@yahoo.fr

Noreddine Boukhatem
n.boukhatem@ump.ma

Jean-Claude Twizere
claude.twizere@uliege.be

Nathalie Meuleman
nathalie.meuleman@bordet.be

Philippe Lewalle
philippe.lewalle@bordet.be

Laurence Lagneaux
laurence.lagneaux@bordet.be

Makram Merimi
makram.merimi.cri@gmail.com

1 Laboratory of Experimental Hematology, Jules Bordet Institute, Université Libre de Bruxelles, Brussels, Belgium

2 Genetics and Immune Cell Therapy Unit, Faculty of Sciences, University Mohammed Premier, Oujda, Morocco

3 Laboratory of Clinical Cell Therapy, Jules Bordet Institute, Université Libre de Bruxelles, Brussels, Belgium

4 Osteoarthritis Research Unit, University of Montreal Hospital Research Center (CRCHUM), Montreal, Canada

5 Institut de Recherche Expérimentale et Clinique (IREC), Laboratory of Pediatric Hepatology and Cell Therapy, Université Catholique de Louvain, Brussels, Belgium

6 Laboratory of Human Pathologies Biology, Faculty of Sciences, Mohammed V University Rabat, Agdal, Rabat, Morocco

7 Laboratory of Viral Interactomes, GIGA Institute, University of Liege, Liege, Belgium

8 Center for Cancer Systems Biology (CCSB), Dana-Farber Cancer Institute, Boston, MA, USA

9 Department of Medicine, University of Montreal, Montreal, Canada