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In vitro antibacterial activity of extracts from Samoan medicinal plants and their effect on proliferation and migration of human fibroblasts

A. Frankova [a], L. Vistejnova [b], T. Merinas-Amo [c], Z. Leheckova [b], I. Doskocil [d], J. Wong Soon [e], T. Kudera [f], F. Laupua [e], A. Alonso-Moraga [c], L. Kokoska [f,*]

[a] *Department of Food Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Praha 6, Suchdol, Czech Republic*
[b] *Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Alej Svobody 1655/76, 323 00, Plzen, Czech Republic*
[c] *Department of Genetics, University of Cordoba, Campus Rabanales, Gregor Mendel Building, 14071, Cordoba, Spain*
[d] *Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Praha 6, Suchdol, Czech Republic*
[e] *Scientific Research Organisation of Samoa, P.O. Box 6597, Apia, Samoa*
[f] *Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Praha 6, Suchdol, Czech Republic*

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ABSTRACT

Ethnopharmacological relevance: The prevalence of different types of chronic wounds, due to the ageing population and increase incidence of diseases, is becoming a worldwide problem. Various medicinal plants used in folk medicine have demonstrated wound healing and antimicrobial properties, and some of these species are currently used in commercial preparations. Despite the well-documented and rich tradition of the use of local herbs for the treatment of skin injuries in Samoan folk medicine, their wound healing potential has not yet been systematically studied.

Aim of the study: Investigation into the *in vitro* antibacterial activity of ethanol extracts from 14 medicinal plants used in Samoan traditional medicine for the healing of wounds, burns and sores, and their effects on the proliferation and migration of human fibroblasts.

Materials and methods: The antibacterial activity of these extracts was tested against pathogens associated with infected skin injuries, using the broth microdilution method. The effect on migration, proliferation and viability of human dermal fibroblasts was evaluated using wound healing scratch assay, cell proliferation assay, and thiazolyl blue tetrazolium bromide cytotoxicity test.

Results: The extracts from *Cerbera manghas, Commelina diffusa, Kleinhovia hospita, Mikania micrantha, Omalanthus nutans, Peperomia pellucida, Phymatosorus scolopendria, Piper graeffei, Psychotria insularum,* and *Schizostachyum glaucifolium* inhibited the growth of *Staphylococcus aureus* at the minimum inhibitory concentration (MIC) of ≥4 μg/mL, whereas *C. manghas* and *P. pellucida* produced the same MIC against both *Escherichia coli* and *Pseudomonas aeruginosa*. Among the antibacterially active species, *C. diffusa*, *K. hospita*, *P. scolopendria*, *P. insularum*, and *S. glaucifolium* did not produce toxicity towards the standard line of normal adult human dermal fibroblasts (IC_{80} > 128 μg/mL). In addition, extracts from *Barringtonia asiatica*, *C. manghas*, *M. micrantha*, *O. nutans*, *P. insularum*, and *Piper graeffei* stimulated significant migration of dermal fibroblasts, while *M. micrantha*, *O. nutans*, and *P. insularum* did not affect cell proliferation at a concentration of 32 μg/mL.

Conclusions: The results suggest that the above-mentioned species of Samoan medicinal plants can be used for the development of new wound healing agents. However, further phytochemical and pharmacological research is needed regarding the isolation and identification of their active constituents.

* Corresponding author.
E-mail address: kokoska@ftz.czu.cz (L. Kokoska).

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1. Introduction

The prevalence of chronic wounds is a rapidly growing problem worldwide, due to increasing health care costs, an ageing population, and a sharp rise in the incidence of diseases such as diabetes and obesity (Martinengo et al., 2019). Similarly, burn injuries are a serious global public health problem associated with high incidences of death and disability, multiple surgical procedures, prolonged hospitalization, and high health care costs (Rowan et al., 2015). Additionally, skin and underlying tissue injuries, known as pressure sores, are the third most expensive disorder after cancer and cardiovascular diseases (Agrawal and Chauhan, 2012). From the pharmacological point of view, the stimulation of dermal fibroblast proliferation, migration and differentiation is a crucial aspect of the wound healing process, which is necessary for maintaining the barrier function of the skin (Portou et al., 2015). Similarly, controlling the microbial load is a vital factor in minimizing infection, and for the treatment of clinically infected wounds and burns. Aerobic or facultative anaerobic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and coliform bacteria, are the primary causes of delayed healing and infection; however, various anaerobic pathogens belonging to genera *Bacteroides*, *Clostridium*, *Cutibacterium*, and *Finegoldia* are also frequently colonizing wounds and burns. Currently, the methicillin-resistant *S. aureus* (MRSA) represents a serious problem, especially in the treatment of chronic wounds. As the development of bacterial resistance to antibiotics continues and controversy regarding the use of topical antiseptics persists, the identification and development of new antimicrobial agents that are cost-efficient, safe, broadly effective, and have a low propensity to induce resistance, is urgently needed (Bowler et al., 2001).

It is well-recorded that various medicinal plants used in folk medicine have demonstrated wound healing and antimicrobial properties in a number of *in vitro*, *in vivo* and clinical studies (Kokoska et al., 2019; Shedoeva et al., 2019). For example, *Aloe vera* has been used for the treatment of wounds, burns, and infections since ancient times. The healing property of *A. vera* is related to its glucomannans content, which stimulate the proliferation of skin cells and improve collagen production and secretion (Hashemi et al., 2015). Currently, *A. vera*-impregnated gauze dressing is commonly applied on postsurgical and newly diagnosed wounds (Adams et al., 2012). *Centella asiatica* is a plant that has been used as a wound healing agent by diverse ancient cultures and tribal groups. The topical application of *C. asiatica* on the injured area of skin results in decreased granulation and scar formation, and increased skin tensile strength. The significant wound healing property of *C. asiatica* led to its commercialization, in the form of topical skin ointments and creams (de Fatima et al., 2008). In addition, various plant-derived antimicrobials that are commonly used for the treatment of skin infections, such as *Melaleuca alternifolia* essential oil, have been discovered based on traditional herbal knowledge (Kokoska et al., 2019). Although plants used for therapeutic purposes are normally assumed to be safe, recent scientific studies have highlighted the toxic effects of many plants used in traditional medicine. Since the toxicity of certain medicinal plants may result in the mutagenicity of human cells, it is essential to test plant materials for their potential adverse effects (Mabona and Van Vuuren, 2013). Considering the above-mentioned, it is obvious that plants used in traditional medicine have great potential for the development of wound healing preparations. However, the pharmacological properties of the majority of these species remain unexplored by modern science.

Samoa, a small South Pacific island country officially known as Independent State of Samoa, has the second largest native flora in Polynesia, consisting of approximately 550 angiosperm species belonging to approximately 300 genera and 95 families. The endemism is typical of Samoan islands, whereas the estimated level of endemic species of flowering plants is about 30% (Whistler, 2002). Although the general knowledge of ancient Samoans on medicinal use of local plants was probably limited, their external application for the healing of skin complaints was very common. Since the earliest times, skin ulcers and infections, as well as infected cuts and wounds, have been some of the most serious health problems in Samoa (Whistler, 1996). Similar to other Pacific regions, wounds inflicted by collisions with reef or rocks are characterized by stubborn healing, and propensity to infection is very frequent (McArthur et al., 2020). Burns are also very common, since open fires are still used to cook food, frequently in open cookhouses where fire and hot pots are easily accessible to young children. Skin sores and ulcers are particularly common in the hot, humid climate of Samoa, and may result from small cuts or insect bites. It is well-documented that there are a number of herbal treatments for wounds and burns in Samoan folk medicine since ancient times (Whistler, 1996). Circumcision has been practiced among Pacificans (Thomson et al., 2006), and herbs were traditionally applied to the circumcision wounds of Samoans (Whistler, 1996). Despite the existence of sporadic reports on the antibacterial effect of Samoan medicinal plants (Norton et al., 1973), their wound healing properties have not been systematically studied. Therefore, it was decided to investigate the *in vitro* effect of extracts from medicinal plants used in Samoan traditional medicine for the healing of wounds, burns and sores, on the proliferation and migration of human dermal fibroblasts, and the growth of bacterial pathogens associated with infected skin injuries.

2. Materials and methods

2.1. Plant materials

Different parts (mainly leaves but also bark, aerial parts and seeds) of 14 medicinal plants were collected from various locations in the Independent State of Samoa during September 2015. The collected samples were subsequently air-dried for several days, and sent to the Czech Republic for further processing and bioactivity testing. Ethnobotany expert, Prof. Kokoska, authenticated the species, and their voucher specimens have been deposited in the herbarium of the Department of Botany and Plant Physiology of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague (Prague, Czech Republic). Plant species were identified using Flora of China (Zhengyi et al., 1994), and through comparison with living specimens grown in the Vailima Botanical Garden (Apia, Samoa). The scientific names of the collected species were reviewed using The Plant List (2013), and their local names were verified with data from literature (Whistler, 1996, 2000). The selection of plants and their collected parts was based on literature regarding their uses in traditional Samoan medicine for the treatment of wounds, burns and sores (Castro and Tsuda, 2001; Whistler, 1996, 2000; WHO, 1998). Since the herbal remedies used in folk medicine for wound healing are expected to prevent and control wound infections (Builders and Builders, 2016), the same indications were also used in the selection of plant species for antibacterial activity testing. For all of the species assayed, the scientific names, families, local names, voucher specimen codes, photographs of collected plant species and herbarium specimen vouchers, GPS coordinates of collection sites, collected parts (plant samples), their uses in folk medicine and previously detected phytochemicals are presented in Table 1.

2.2. Preparation of plant extracts

Although the most common form of folk Samoan treatment is crushed fresh plant material applied directly to the wound (Whistler, 1996), ethanol was selected for the extraction of plant samples, since it is an efficient solvent for herbal drugs with a well-established tradition in herbal medicine (Kelber et al., 2017). With the aim of preventing possible loss or changes of active constituents due to storage of plant samples, the extraction was performed immediately after their arrival in the Czech Republic. Each dried plant sample was finely ground into a powder using a Grindomix GM100 knife mill (Retsch, Haan, Germany),

and 15 g was then extracted in 450 mL of 80% ethanol (Penta, Prague, Czech Republic) for 24 h at room temperature, using an orbital shaker (GFL3005, GFL, Burgwedel, Germany). Extracts were subsequently filtered and concentrated using a rotary vacuum evaporator (R-200, Buchi Labortechnik, Flawil, Switzerland) *in vacuo* at 40 °C. The dried residue was finally diluted in 100% dimethylsulfoxide (DMSO) (Penta, Prague, Czech Republic) to obtain stock solutions with a final concentration of 51.2 mg/mL, and stored at -20 °C until their use. Yields (%) of the dried residues are shown in Table 1.

2.3. Bacterial strains and media

The *in vitro* growth-inhibitory activities of the ethanol extracts were determined against main representatives of bacterial pathogens present in acute and chronic wounds and burns (Bowler et al., 2001), including 5 anaerobic and 3 aerobic bacterial species dominated by 16 strains of *S. aureus*. Standard American Type Culture Collection (ATCC) strains, namely *Bacteroides fragilis* ATCC 25285, *Escherichia coli* ATCC 25922, *Cutibacterium acnes* ATCC 11827, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC (25923, 29213, 33591, 33592, 43300, and BAA 976) were obtained from Oxoid (Basingstoke, UK). The German Resource Centre for Biological Material (DSMZ) (Braunschweig, Germany) provided the cultures of *Clostridium difficile* DSMZ 12056, *Clostridium perfringens* DSMZ 11778, and *Finegoldia magna* DSMZ 2974. Ten clinical isolates of *S. aureus* (SA1–10) were provided by Motol University Hospital (Prague, Czech Republic), and their identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry, according to Rondevaldova et al. (2018). With the exception of clostridial strains stored in cooked meat medium (Oxoid, Basingstoke, UK) at room temperature, all cultures of aerobic bacteria were maintained using Mueller-Hinton agar (Oxoid, Basingstoke, UK), and stored at 4 °C until use. Mueller-Hinton broth (Oxoid, Basingstoke, UK) was used as a growth medium for all tested aerobic bacteria (cation-adjusted for *S. aureus* strains), whereas anaerobes were cultured in Wilkins-Chalgren broth (Oxoid, Basingstoke, UK) enriched with 5 g/L soya peptone and 0.5 g/L cysteine, under anaerobic conditions within a Whitley A35 Anaerobic Workstation (Don Whitley Scientific, West Yorkshire, UK). The pH of the media used was equilibrated to a final value of 7.6 using Trizma base (Sigma-Aldrich, Prague, Czech Republic).

2.4. Preparation and isolation of dermal fibroblasts cultures

Cytotoxicity of the extracts was evaluated against a standard cell line (CC-2511) of normal human dermal fibroblasts derived from the dermis of adult skin (NHDF-Ad). The cells were cultured in fibroblast growth medium 2 (FGM 2) supplemented with insulin, human fibroblast growth factor-basic, gentamicin sulphate-amphotericin (GA-1000) (each at a concentration of 0.001%) and 2% foetal bovine serum (FBS). Cultures were incubated in 5% CO_2 at 37 °C. Culture media were replenished every 2nd day, and cells passages every 3rd day. All chemicals and cultures were purchased from Lonza (Basel, Switzerland).

Cell proliferation and wound healing assays were performed using normal human dermal fibroblasts isolated from the facial skin (NHDF-FS) of 6–10 year-old female donors. The skin samples were removed during cosmetic plastic surgery at the Department of Plastic Surgery of the University Hospital in Pilsen (Czech Republic), under informed agreements of the donors and after approval by the ethical committee of the University Hospital in Pilsen (Pilsen, Czech Republic). The guidelines specified within the Declaration of Helsinki were followed. NHDF-FS were subsequently cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 0.015% L-glutamine (all three ingredients purchased from Thermo Fisher Scientific, Waltham, MA, USA), 1% nonessential amino acids, 100 U/mL of penicillin and 0.1 mg/mL of streptomycin (all tree chemicals obtained from Biosera, Nuaille, France), and incubated in 5% CO_2 at 37 °C. NHDF-FS from the 2nd to 9th passages were used for cell proliferation and wound healing experiments.

2.5. Broth microdilution method

Minimum inhibitory concentrations (MICs) were determined using the standard broth microdilution method in 96-well microtiter plates, according to the approved guidelines and recommendations for susceptibility testing of aerobic (Clinical and Laboratory Standards Institute, 2009) and anaerobic (Hecht, 1999) bacteria, whilst implementing slight modifications proposed by Cos et al. (2006) for the effective assessment of the anti-infective potential of natural products. Serial dilutions of the extracts were prepared in an appropriate growth medium (100 μL), ranging from 4 to 512 μg/mL, using the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, Switzerland) and a manual multichannel pipette (Eppendorf, Wesseling-Berzdorf, Germany) for aerobic and anaerobic bacteria, respectively. All bacterial cultures were diluted to contain 1.5×10^8 CFU/mL, and the plates were inoculated with the bacterial suspension using a 96-pin multi-blot replicator (National Institute of Public Health, Prague, Czech Republic). Microplates were then incubated for 24 h at 37 °C. The plates inoculated with anaerobes were prepared and incubated within a Whitley A35 Anaerobic Workstation (Don Whitley Scientific, Shipley, UK). Bacterial growth was determined through absorbance measurement using a Cytation 3 Imaging Reader (BioTek, Vermont, USA) at 405 nm. The lowest extract concentration showing ≥80% reduction of microbial growth compared to the positive growth control was considered as the MIC. Gentamicin and oxacillin (Sigma-Aldrich, Prague, Czech Republic), antibiotics commonly recommended for the treatment of wounds (Bowler et al., 2001), were dissolved in distilled water and used as positive controls. *S. aureus* 29213 was used as a control strain for antibiotic susceptibility testing, and other staphylococcal strains were identified as MRSA when the oxacillin MIC was ≥4 mg/L (Clinical and Laboratory Standards Institute, 2009). The solvents used (DMSO and ethanol) did not inhibit bacterial growth at the concentration tested (≤1%). All tests were performed as three independent experiments, each carried out in triplicate, and the results are presented as median/modal values. According to the widely accepted norm in MIC testing, the mode and median were used for the final value calculation when the triplicate endpoints were within the two- and three-dilution range, respectively.

2.6. Cytotoxicity assay

A slightly modified method was used to test cell viability, based on the metabolization of thiazolyl blue tetrazolium bromide (MTT, obtained from Sigma-Aldrich, Prague, Czech Republic) to blue formazan by mitochondrial dehydrogenases in living cells, previously described by Mosmann (1983). Briefly, NHDF-Ad were seeded in 96-well plates at a density of 5×10^3 cells per well. After 24 h, the cells were treated with serially diluted plant extracts in concentrations of 4–128 μg/mL for a further 72 h. Thereafter, the medium containing the extracts was replaced with fresh FGM 2 medium containing MTT reagent (1 μg/mL), and plates were incubated for an additional 2 h at 37 °C. Subsequently, the produced formazan crystals were dissolved in 100 μL of DMSO, and the absorbance was then measured at 555 nm using a Tecan Infinite M200 spectrometer (Tecan Group, Mannedorf, Switzerland). The results of the cytotoxicity effect were expressed as half maximal inhibitory concentrations (IC_{50}) in μg/mL. The levels of cytotoxic effect were classified according to the WHO (2020) as cytotoxic (IC_{50} < 2.00 μg/mL), moderately cytotoxic (IC_{50} = 2.00–89.00 μg/mL), and not cytotoxic (IC_{50} > 90.00 μg/mL). Furthermore, the 80% inhibitory concentration of proliferation (IC_{80}) was calculated as equivalent to the MIC endpoint, usually defined as 80% bacterial growth inhibition (Kokjohn et al., 2003). Fluorouracil (Sigma-Aldrich, Prague, Czech Republic), a medication used topically for actinic keratosis, basal cell carcinoma, and skin warts (Moore, 2009), was tested as the positive cytotoxic control within a concentration range of 0.0625–128 μg/mL. Results are

Table 2
Antibacterial activity of Samoan plant extracts.

Bacterium/ strain	Plant species and positive antibiotic controls*/minimum inhibitory concentrations (MICs, μg/mL)															
	Barringtonia asiatica	*Cerbera manghas*	*Commelina diffusa*	*Inocarpus fagifer*	*Kleinhovia hospita*	*Mikania micrantha*	*Omalanthus nutans*	*Peperomia pellucida*	*Phymatosorus scolopendria*	*Piper graeffei*	*Premna serratifolia*	*Psychotria insularum*	*Schizostachyum glaucifolium*	*Solenostemon scutellarioides*	Oxacillin*	Gentamicin*
Standard strains																
B. fragilis	>512	>512	>512	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	16	128
C. difficile	>512	>512	>512	512	512	>512	>512	>512	>512	>512	>512	>512	>512	512	32	64
C. perfringens	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	2	128
C. acnes	512	>512	128	>512	>512	256	256	>512	>512	256	512	128	256	256	8	64
E. coli	>512	**4**	256	>512	>512	>512	512	**4**	>512	>512	>512	>512	>512	>512	512	1
F. magna	>512	512	512	256	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	8	32
P. aeruginosa	>512	**4**	512	>512	>512	>512	**4**	**4**	>512	>512	>512	>512	>512	>512	>512	1
Staphylococcus aureus standard strains																
BAA 976	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	512	512	16	>512
SA 25923	512	>512	>512	>512	>512	512	>512	>512	512	>512	>512	128	>512	>512	0.5	0.25
SA 29213	512	>512	>512	>512	128	>512	512	512	512	>512	>512	512	>512	>512	0.5	0.5
SA 33591	>512	>512	>512	>512	>512	512	>512	>512	512	>512	>512	128	512	>512	256	16
SA 33592	512	>512	256	>512	>512	>512	256	**64**	256	**4**	>512	**4**	**4**	>512	64	0.5
SA 43300	512	>512	>512	>512	512	>512	256	>512	>512	>512	>512	512	512	>512	64	1
Staphylococcus aureus clinical isolates																
SA1	>512	>512	>512	>512	256	512	>512	>512	512	**4**	512	512	**4**	256	32	8
SA2	>512	>512	>512	>512	>512	512	>512	>512	256	>512	512	512	512	512	128	16
SA3	512	**4**	**4**	>512	**4**	**4**	**4**	**4**	**4**	**4**	512	>512	**4**	512	64	8
SA4	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	>512	512	256	8
SA5	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	>512	512	4	8
SA6	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	512	>512	>512	>512	128	512
SA7	512	>512	>512	>512	**4**	>512	512	>512	>513	>514	>515	>516	>517	>518	0.5	256
SA8	>512	**4**	**4**	>512	**4**	**64**	**4**	**4**	**4**	**4**	512	>512	**4**	512	32	8
SA9	512	**4**	**4**	>512	256	**4**	**4**	**4**	**4**	**4**	512	>512	**4**	512	1	8
SA10	512	**4**	**4**	>512	512	**4**	**4**	**4**	**4**	**4**	512	128	**4**	512	0.25	8

B. fragilis: *Bacteroides fragilis*, *C. difficile*: *Clostridium difficile*, *C. perfringens*: *Clostridium perfringens*, *C. acnes*: *Cutibacterium acnes*, *E. coli*: *Escherichia coli*, *F. magna*: *Finegoldia magna*, *P. aeruginosa*: *Pseudomonas aeruginosa*; numbers in bold indicate significant antibacterial effect (MIC < 100 μg/mL) of plant extracts (Kokoska et al., 2019).

expressed as the mean ± standard deviation (SD) from three independent repetitions, each of which was performed in duplicate.

2.7. Wound healing scratch assay

The analysis of cell migration was performed using a scratch assay according to Liang et al. (2007). The isolated NHDF-FS were seeded on 6-well plates at a density of cells 23 000/cm^2 and cultivated at 37 °C in 5% CO_2 overnight. The next day, the cell monolayers were scratched using a 10 μL pipette tip to create four crosses (wound area) per well. After scratching, the culture medium was aspirated and detached cells were washed using phosphate-buffered saline (PBS). The cell monolayers were subsequently treated with 2 mL of a new culture medium containing 32 μg/mL of the extract, and incubated for 24, 48 and 72 h.

Cell migration into the wounded area was monitored and photographed using phase contrast microscopy (Olympus IX83, Olympus Corporation, Tokyo, Japan) under a 4 × magnification objective immediately after the treatment and then 24, 48 and 72 h later. Final images were analysed with TScratch software (CSELab, Zurich, Switzerland). The area without cells (wounded area) detected by the software was expressed as a portion of the total picture area (=100%). The results are expressed as the mean ± SD, and were calculated as a ratio of the wound area at 24, 48 and 72 h to that of the wound area at 0 h. All treatments were performed in a serum-free medium to eliminate cell proliferation. Serum-free medium was also applied as a control in all experiments. Assays were performed in three independent experiments (each done in triplicate), and NHDF-FS from different donors were used for each experiment.

2.8. Cell proliferation assay

Cell proliferation was estimated through double-stranded (ds) DNA detection using the intercalation reagent PicoGreen (CyQuant Cell Proliferation Assay Kit, Thermo Fisher Scientific, Waltham, USA), which after binding to dsDNA, becomes strongly fluorescent, and is widely established in cell proliferation assays (Singer et al., 1997; Vojtova et al., 2019). NHDF-FS were seeded on 96-well plates at a density of cells 9000/cm^2 in 150 μL of culture medium per well, and incubated in 5% CO_2 at 37 °C overnight. The next day, the old culture medium was replaced with 150 μL of a new culture medium containing 32 μg/mL of the extract, and incubated for 24, 48 and 72 h. NHDF-FS in culture media were used as the untreated control. All culture media were enriched with 10% FBS. At each time point, the culture medium was aspirated, NHDF-FS were washed with PBS, and stored at -80 °C until analysis. Before analysis, NHDF-FS were thawed, and 100 μL of PicoGreen in TE buffer was added to each well, followed by a 5 min incubation at room temperature in dark. Fluorescence was measured at excitation (485 nm) and emission (528 nm) wavelengths by a microplate reader (Synergy HT, Biotek, Vermont, USA). All tests were performed as three independent experiments, each carried out in triplicate, and NHDF-FS from different donors were used for each experiment. The results are expressed as the mean ± SD, and represent the relative ratio (%) of the number of cells in the treated samples at a given time to the number of the cells in the untreated control at the beginning of the experiment (time 0).

2.9. Statistical analysis and calculations

In case of cytotoxicity assay, IC_{50} and IC_{80} values were calculated using the 4-parameter logistic model using Magellan software (Tecan Group, Mannedorf, Switzerland). Statistical analysis for cell proliferation and wound healing assays was performed in Statistica Version 12 software (TIBCO Software, Palo Alto, USA). The data normality was tested by the Shapiro-Wilk test, and for the comparison of treated-untreated groups, Wilcoxon match paired test was performed. The differences were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Antibacterial activity

As far as the anti-staphylococcal action of Samoan plants is concerned, the ethanol extracts of *Cerbera manghas*, *Commelina diffusa*, *Kleinhovia hospita*, *Mikania micrantha*, *Omalanthus nutans*, *Peperomia pellucida*, *Phymatosorus scolopendria*, *Piper graeffei*, *Psychotria insularum*, and *Schizostachyum glaucifolium* inhibited the growth of certain strains of *S. aureus* at the lowest concentration tested (4 μg/mL). With the exception of *P. insularum*, all of these extracts also exhibited lower MIC values against certain strains of *S. aureus* than both antibiotic controls, oxacillin and gentamicin. Other extracts, namely *Barringtonia asiatica*, *Inocarpus fagifer*, *Premna serratifolia*, and *Solenostemon scutellarioides* possessed only moderate or weak anti-staphylococcal effects. Interestingly, clinical isolates (especially SA3, 8–10) were more susceptible than standard strains, including those resistant to methicillin. Although other bacteria affecting wound healing, namely *B. fragilis*, *C. difficile*, *C. perfringens*, *C. acnes*, and *F. magna*, were resistant to all plants tested (MICs ≥ 128 μg/mL), the extracts from *C. manghas*, and *P. pellucida* produced a strong inhibitory effect against both *E. coli* and *P. aeruginosa* (MIC = 4 μg/mL). Table 2 presents the complete results of the antibacterial effects of the Samoan plant extracts against pathogens associated with infected skin injuries.

The results correspond with the data summarized in the recent review on the chemical and biological properties of *P. pellucida*, showing a broad spectrum of antimicrobial effects of the plant, including *in vitro* growth-inhibitory actions against *S. aureus* and *P. aeruginosa* (Alves et al., 2019). Although the seed extract of *C. manghas*, as well as leaf and seed oils of *K. hospita*, were reported to produce antibacterial effects (Ahmed et al., 2008; Dey et al., 2019), this is the first report on the antibacterial activity of their leaves and bark, respectively. Since flavonoids such as nicotiflorin and rutin previously detected in the leaves of *C. manghas* (Shen et al., 2007) are known to produce antimicrobial effects (Soberon et al., 2010), they can contribute to the antibacterial effect of extracts tested. Cycloartane triterpenoids and triterpenoid alkaloids have recently been isolated from the leaves of *K. hospita* (Rahim et al., 2018); however, the chemical composition of its bark is still unknown. In correspondence with the present results, anti-staphylococcal and antibacterial effects of *C. diffusa*, *M. micrantha*, and *P. scolopendria*, have been previously reported (Ghosh et al., 2008; Huish et al., 2014; Khan et al., 2011; Lentz et al., 1998; Mensah et al., 2006). It has been suggested that the antibacterial properties of *M. micrantha* can be attributed to sesquiterpenes, particularly mikanolide and dihydromikanolide, previously isolated from its leaves (Bakir et al., 2004; Li et al., 2013). Since the phenolic compounds, such as flavonoids and tannins, detected in ethanol extract from the aerial part of *P. scolopendria* (Sujatha et al., 2018) together with terpenoids identified in the aerial part of *C. diffusa* (Malarvizhi et al., 2019) are known to produce antibacterial effects (Kokoska et al., 2019), it is possible to suggest that compounds belonging to these phytochemical groups previously detected in both species may contribute to their antibacterial actions. This study is the first to report on antibacterial activity of *O. nutans*, *P. graeffei*, *P. insularum*, and *S. glaucifolium*.

3.2. Toxicity to standard line of NHDF-Ad

The majority of tested plant species, namely *C. diffusa*, *I. fagifer*, *K. hospita*, *P. scolopendria*, *P. serratifolia*, *P. insularum*, *S. glaucifolium* and *S. scutellarioides*, did not exhibit significant cytotoxicity against NHDF-Ad (IC_{50} ≥ 113.86 μg/mL). With the exception of *C. manghas*, which was found to be highly cytotoxic (IC_{50} = 0.13 μg/mL), the remaining plants (*B. asiatica*, *M. micrantha*, *O. nutans*, *P. pellucida* and *P. graeffei*) exhibited only moderate toxicity with IC_{50} values ranging from 14.24 to 88.57 μg/mL. In the case of IC_{80} values, the highest cytotoxicity was also observed for the extract of *C. manghas* (IC_{80} = 6.36 μg/mL), followed by

Table 3
Cytotoxic activity of Samoan plant extracts.

Plant species and positive cytotoxic control*	Inhibitory concentration (IC) ± standard deviation (μg/mL)	
	IC_{50}	IC_{80}
Barringtonia asiatica	14.24 ± 2.74	21.01 ± 1.8
Cerbera manghas	0.13 ± 0.02	6.36 ± 0.93
Commelina diffusa	> **128**	>128
Inocarpus fagifer	> **128**	>128
Kleinhovia hospita	> **128**	>128
Mikania micrantha	88.57 ± 1.84	>128
Omalanthus nutans	69.94 ± 2.08	>128
Peperomia pellucida	63.52 ± 18.51	>128
Phymatosorus scolopendria	> **128**	>128
Piper graeffei	30.63 ± 1.31	>128
Premna serratifolia	> **128**	>128
Psychotria insularum	**113.86 ± 3.94**	>128
Schizostachyum glaucifolium	> **128**	>128
Solenostemon scutellarioides	> **128**	>128
Fluorouracil*	>128	>128

IC_{50}: 50% inhibitory concentration; IC_{80}: 80% inhibitory concentration; numbers in bold indicate no cytotoxic effect (IC_{50} > 90.00 μg/mL) of plant extracts (WHO, 2020).

B. asiatica (IC_{80} = 21.01 μg/mL). All other extracts' IC_{80} values were higher than 128 μg/mL. Complete data on the cytotoxicity of Samoan plant extracts are shown in Table 3.

The results report high cytotoxicity of *C. manghas*, which corresponds with previously published papers on the toxicity of leaf extract to human leukaemia cells *in vitro* (Masuda et al., 2002) and to mice *in vivo* (Hien et al., 1991). Various classes of compounds, such as olivil glucosides, normonoterpenoids and megastigmanes, have been previously isolated from the leaves of the species (Shen et al., 2007); however, their cytotoxic effects are not known. In this study, the extract of *P. pellucida* exhibited moderate toxicity against normal human dermal fibroblasts. Although cytotoxicity of the plant has been extensively studied using various carcinogenic cell lines, experiments with healthy cells are limited. In one of these studies, the methanol extract of *P. pellucida* aerial plant showed no toxicity against normal human kidney cells (Alves et al., 2019). Despite previous reports that crude extracts and sesquiterpene lactones from *M. micrantha* exhibited cytotoxicity against various human cancer lines (Barbhuiya and Devi, 2019; Dou et al., 2014; Rios et al., 2014), this is the first reporting of the moderate toxicity of plant extracts to healthy human cell lines. Although several classes of compounds isolated from the bark and leaves (triterpenes) of *B. asiatica* (Ragasa et al., 2014), leaves (triterpenoids, flavonoids and quinones) of *K. hospita* (Arung et al., 2012; Mo et al., 2014), wood (lignans, terpenoids and steroids) of *P. serratifolia* (Biradi and Hullatti, 2017; Woo et al., 2019), were tested for their toxicity to various human cancer cell lines, this is the first cytotoxicological study using extracts from seeds and bark of the above-mentioned species, respectively. Moreover, this is the first report on the evaluation of cytotoxicity of *C. diffusa*, *I. fagifer*, *O. nutans*, *P. scolopendria*, *P. graeffei*, *P. insularum*, *S. glaucifolium* and *S. scutellarioides*.

3.3. Effect of NHDF-FS on migration and proliferation

In comparison with the untreated control, a concentration of 32 μg/mL of *B. asiatica, C. manghas, M. micrantha, O. nutans, P. insularum,* and *P. graeffei* extracts produced statistically significant ($p < 0.05$) stimulation of NHDF-FS migration to the wound area after 24, 48 and 72 h. In addition, extracts of *C. diffusa, I. fagifer, K. hospita, P. pellucida, S. glaucifolium,* and *S. scutellarioides* supported cell migration in at least one measured timepoint. In contrast, extracts of *P. scolopendria* and *P. serratifolia* had no effect on NHDF-FS migration (Fig. 1). Similarly, as in case of toxicity response of the standard line of NHDF-Ad, the majority of tested plant species (*C. diffusa*, *I. fagifer*, *K. hospita*, *M. micrantha*, *O. nutans*, *P. pellucida*, *P. scolopendria*, *P. insularum*, *P. serratifolia*, *S. glaucifolium*, and *S. scutellarioides*) did not affect proliferation of NHDF-FS. At the tested concentration (32 μg/mL), only extracts from *B. asiatica*, *C. manghas* and *P. graeffei* exhibited certain

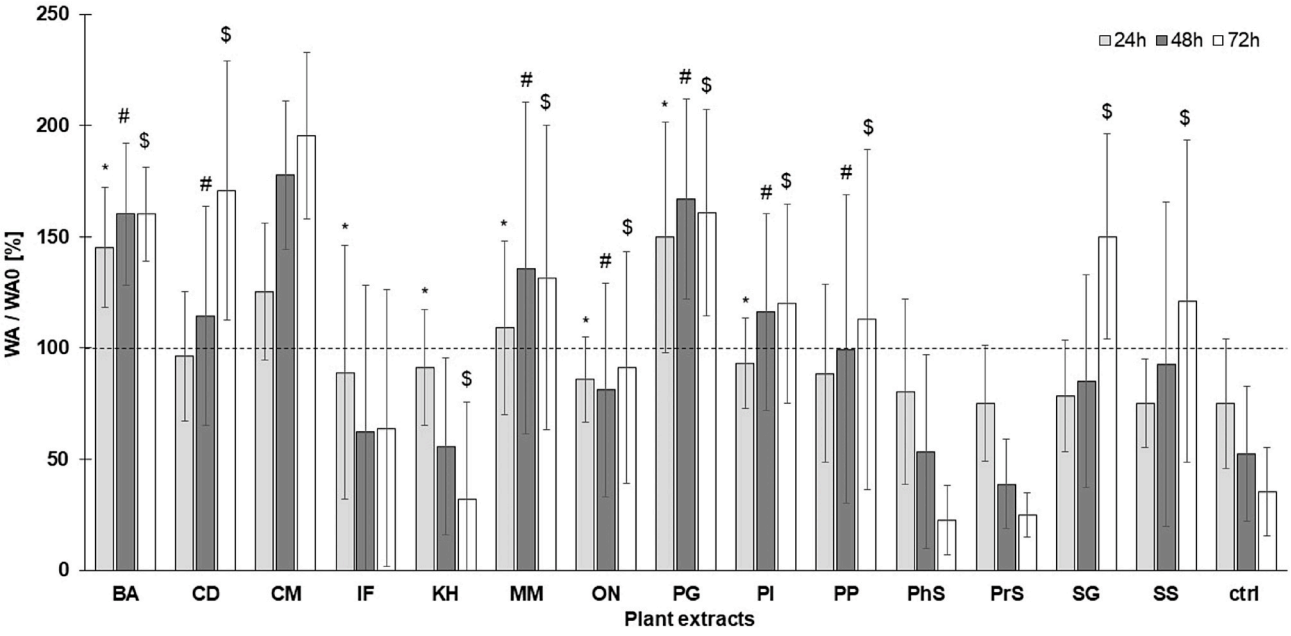
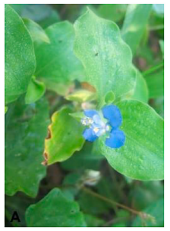
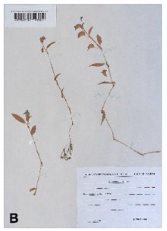
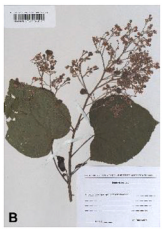


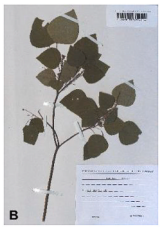
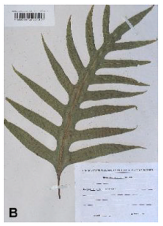
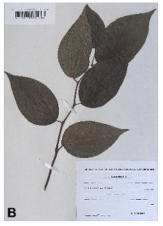
Fig. 1. Effect of Samoan plant extract on migration of normal human dermal fibroblasts isolated from the facial skin (NHDF-FS). BA: *Barringtonia asiatica*, CD: *Commelina diffusa*, CM: *Cerbera manghas*, IF: *Inocarpus fagifer*, KH: *Kleinhovia hospita*, MM: *Mikania micrantha*, ON: *Omalanthus nutans*, PG: *Piper graeffei*, PI: *Psychotria insularum*, PP: *Peperomia pellucida*, PhS: *Phymatosorus scolopendria*, PrS: *Premna serratifolia*, SG: *Schizostachyum glaucifolium*, SS: *Solenostemon scutellarioides*. The cell migration was examined at concentration 32 μg/mL and expressed as a relative ratio (%) of wound area (WA) at 24, 48 and 72 h to wound area in the beginning of the experiment (WA0). The results are expressed as mean ± standard deviation of three independent experiments (each done in triplicate), whereas NHDF-FS of different donor were used for each experiment. The symbols *, #, and $ indicate statistically significant difference ($p < 0.05$) between treated samples and untreated control (ctrl) at time 24, 48 and 72 h, respectively.

Table 1
Ethnobotanical and phytochemical data on Samoan medicinal plants.

Latin name [Family]	Photographs		Collection site (GPS coordinates)	Local name	Voucher specimen	Tested part	Extract yield (%)	Ethnomedicinal use	Chemical composition
Barringtonia asiatica (L.) Kurz [*Lecythidaceae*]			13.8657247S, 171.7597639W	Futu	2403KBFR0	Seed	13.9	Skin sores (Whistler, 2000)	Oleane glycosides, saponins, triterpenes (Burton et al., 2003; Herlt et al., 2002; Ragasa et al., 2011)
Cerbera manghas L. [*Apocynaceae*]			13.8490414S, 171.7751425W	Leva	2405KBFR0	Leaf	22.2	Skin sores and infections (Whistler, 2000)	Cardenolide glycosides, flavonoids, iridoids, lactones, lignans, sterols, terpenoids (Abe et al., 1996; Jeong, 2014; Shen et al., 2007; Zhang et al., 2010)
Commelina diffusa Burm.f. [*Commelinaceae*]			13.5369394S, 172.3952458W	Mau'utoga	2406KBFR0	Leaf	9.7	To staunch bleeding of cuts (Whistler, 2000)	Flavonoids, sterols, terpenoids (Hamad et al., 2019; Malarvizhi et al., 2019)
Inocarpus fagifer (Parkinson) Fosberg [*Leguminosae*]			13.9841172S, 171.7158883W	Ifi	2415KBFR0	Leaf	11.9	Wounds (WHO, 1998)	No reports
Kleinhovia hospita L. [*Malvaceae*]			13.8968333S, 171.5730494W	Fu'afu'a	2416KBFR0	Bark	15.2	Cuts and wounds, to staunch bleeding (Whistler, 2000, 1996)	No reports
Mikania micrantha Kunth [*Compositae*]			13.9954850S, 171.9223325W	Fue saina	2419KBFR0	Leaf	23.1	Wounds, to staunch bleeding and prevent	Cadinane sesquiterpenoids, phenolics, sesquiterpene lactones, monoterpenes (Bakir

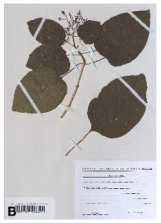
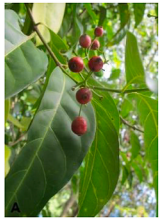
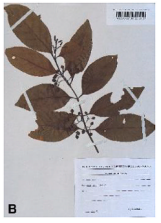
(*continued on next page*)

Table 1 (*continued*)

Latin name [Family]	Photographs		Collection site (GPS coordinates)	Local name	Voucher specimen	Tested part	Extract yield (%)	Ethnomedicinal use	Chemical composition
								infection (Whistler, 2000, 1996)	
									et al., 2004; Bravo-Monzon et al., 2014; Dong et al., 2017; Li et al., 2013; Rios et al., 2014; Zhang et al., 2019)
Omalanthus nutans (G. Forst.) Guillaumin [*Euphorbiaceae*]			13.9972500S, 171.7168467W	Mamala, Fanuamamala	2423KBFR0	Leaf	25.1	Circumcision wounds (Whistler, 2000, 1996; WHO, 1998)	No reports
Peperomia pellucida (L.) Kunth [*Piperaceae*]			13.9856886S, 171.9208125W	Vao vai	2424KBFR0	Aerial part	2.8	Boils (Whistler, 2000)	Alkaloids, essential oil (phenylpropanoids and sesquiterpenes), flavonoids, lignans, quinones, saponins, sterols, tannins, triterpenoids (Alves et al., 2019)
Phymatosorus scolopendria (Burm. f.) Pic. Serm. [*Polypodiaceae*]			13.8290075S, 171.7667367W	Lau maga	2425KBFR0	Leaf	22.6	Hard-to-cure wounds (Whistler, 2000, 1996; WHO, 1998)	Ecdysteroids, phenolic compounds (Snogan et al., 2007; Sujatha et al., 2018)
Piper graeffei Warb. [*Piperaceae*]			13.8997278S, 172.0259856W	Fue manogi	2427KBFR0	Leaf	16.6	Infected wounds (Whistler, 2000)	No reports
Premna serratifolia L. [*Lamiaceae*]			13.8296856S, 171.7547908W	Aloalo	2431KBFR0	Bark	15.5	Wounds and sores (Castro and Tsuda, 2001; Whistler, 2000)	Furofuran lignans, iridoid glycosides, sterols, triterpenes (Yadav et al., 2013)

(*continued on next page*)

Table 1 (*continued*)

Latin name [Family]	Photographs		Collection site (GPS coordinates)	Local name	Voucher specimen	Tested part	Extract yield (%)	Ethnomedicinal use	Chemical composition
Psychotria insularum A. Gray [*Rubiaceae*]			13.8657247S, 171.7597639W	Matalafi	2433KBFR0	Leaf	21.9	Infected wounds (Whistler, 2000)	No reports
Schizostachyum glaucifolium (Rupr.) Munro [*Poaceae*]			13.8560900S, 171.7732883W	‘Ofe	2435KBFR0	Leaf	11.5	Burns (Whistler, 2000)	No reports
Solenostemon scutellarioides (L.) Codd [*Lamiaceae*]			13.8930631S, 171.5806925W	Pate	2436KBFR0	Leaf	12.8	Skin sores (Whistler, 2000)	Diterpenoids (Cretton et al., 2018)

A: collected plant species, B: herbarium specimen voucher (original photos by L. Kokoska).

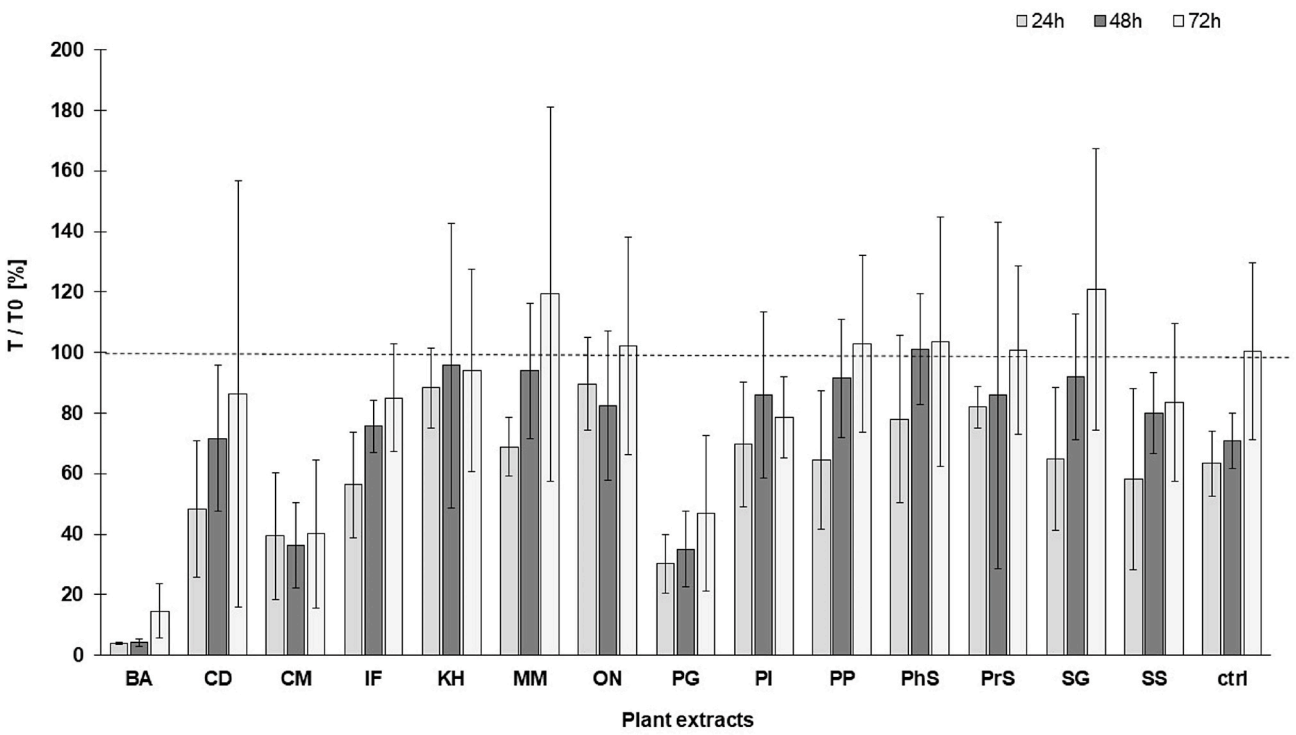


Fig. 2. Effect of Samoan plant extracts on proliferation of normal human dermal fibroblasts isolated from the facial skin (NHDF-FS). BA: *Barringtonia asiatica*, CD: *Commelina diffusa*, CM: *Cerbera manghas*, IF: *Inocarpus fagifer*, KH: *Kleinhovia hospita*, MM: *Mikania micrantha*, ON: *Omalanthus nutans*, PG: *Piper graeffei*, PI: *Psychotria insularum*, PP: *Peperomia pellucida*, PhS: *Phymatosorus scolopendria*, PrS: *Premna serratifolia*, SG: *Schizostachyum glaucifolium*, SS: *Solenostemon scutellarioides*. The cell proliferation was examined at concentration 32 μg/mL and expressed as a relative ratio (%) of the number of cells in treated samples in given time (T) 24, 48 and 72 h to number of the cells in untreated control (ctrl) in the beginning of the experiment (T0). The results are expressed as mean ± standard deviation of three independent experiments (each done in triplicate), whereas NHDF-FS of different donor were used for each experiment.

toxicity towards NHDF-FS cells (Fig. 2).

In a study by Nurdiana et al. (2013) it was reported that the topical application of an ethanol extract from *M. micrantha* leaves on a diabetic excision wound in alloxan-induced male diabetic rats successfully improved the percentage of wound contraction, and altered the arrangement of granulation on the wound site, compared to the other treatments. The present study findings showed that the wound healing properties of *P. graeffei* and *P. pellucida* can be supported by the results from experiments with other species of both genera. For example, the methanol leaf extract of *Piper betle* increased the healing rate of scratch wounds and proliferation of murine fibroblasts *in vitro*, and promoted wound healing in both burn wound and excision wound models *in vivo* (Lien et al., 2015). Similarly, significant wound-healing activity was detected for extracts from the aerial part of *Peperomia galioides* in experiments with mice (Villegas et al., 1997), and epi-α-bisabolol was identified as the component responsible for the pharmacological action of the plant (Villegas et al., 2001). With the exception of *M. micrantha*, the *in vitro* effect of all of the above-mentioned plants on the proliferation and migration of human dermal fibroblasts is reported for first time in this study.

4. Conclusion

In this study evaluating the *in vitro* activity of 14 ethanol extracts from Samoan medicinal plants traditionally used for wound healing, *C. manghas*, *C. diffusa*, *K. hospita*, *M. micrantha*, *O. nutans*, *P pellucida*, *P. scolopendria*, *P. graeffei*, *P. insularum*, and *S. glaucifolium* produced significant growth-inhibitory effects against *S. aureus*, whereas *C. manghas* and *P. pellucida* also inhibited both *E. coli* and *P. aeruginosa*. Among the antibacterial active species, *C. diffusa*, *K. hospita*, *P. scolopendria*, *P. insularum*, and *S. glaucifolium* did not produce toxicity towards standard lines of NHDF-Ad. In addition, extracts from *B. asiatica*, *C. manghas*, *M. micrantha*, *O. nutans*, *P. insularum*, and *P. graeffei* stimulated significant migration of NHDF-FS, while *M. micrantha*, *O. nutans*, and *P. insularum* did not affect cell proliferation. Moreover, the extract from *P. insularum* showed effects combining a certain level of antibacterial action and stimulation of NHDF-FS migration, as well as safety to skin cells. The results suggest that extracts from Samoan medicinal plants are promising materials for further research and development of new plant-derived wound healing agents. However, further phytochemical and pharmacological research will be needed for the isolation and identification of their active constituents.

Author contributions

Frankova A. performed and coordinated antimicrobial activity testing and its related data analysis, and drafted the manuscript; Vistejnova L. performed and coordinated cell proliferation and wound healing scratch experiments and their related statistical analysis; Merinas-Amo T. prepared the plant extracts, and performed the anti-staphylococcal testing; Leheckova Z. participated in the assessment of cell proliferation and wound healing effects; Doskocil I. conducted the cytotoxicity assays and their related data analysis; Wong Soon J. analysed and verified the data on the ethnobotanical use of plants; Kudera T. collected the plant materials and processed the voucher specimens; Laupua F. participated in the collection of plant materials; Alonso-Moraga A. coordinated the cytotoxicity analysis; Kokoska L. conceptualized and coordinated the whole study, provided the botanical identification of plant samples, and finalised the manuscript; all authors have read and approved the final manuscript.

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