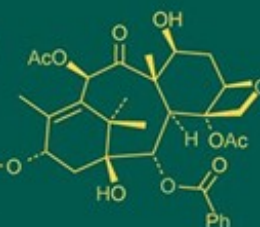
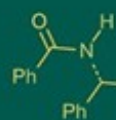
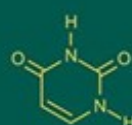
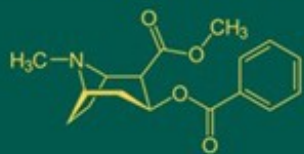


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Evaluation of in vitro cytotoxicity of *Asphodelus tenuifolius* and *Leptadenia pyrotechnica* extracts in equine wound healing

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ABSTRACT

The study was conducted at the ICAR-National Research Centre on Equines, Equine Production Campus (NRCE-EPC), Bikaner, to comparatively evaluate the in vitro cytotoxicity of petroleum ether, methanolic, and aqueous extracts of *Asphodelus tenuifolius* and *Leptadenia pyrotechnica* using equine dermal fibroblasts. Cytotoxicity was assessed using the MTT assay following exposure to two-fold serial concentrations (100-0.457 µg/ml) for 72 h. Cell viability varied in an extract- and concentration-dependent manner. For *A. tenuifolius*, the non-cytotoxic concentrations were 1.23 µg/ml for the petroleum ether extract and 0.14 µg/ml for the methanolic extract, whereas the aqueous extract exhibited comparatively greater cytotoxicity. For *L. pyrotechnica*, 3.70 µg/ml of the petroleum ether extract and 0.05 µg/ml of both methanolic and aqueous extracts were considered suitable concentrations. Overall, the findings demonstrate differential cytotoxicity among the extracts and identify suitable concentration ranges for further evaluation of their potential wound-healing effects in equine dermal fibroblasts

Keywords: Cytotoxicity, MTT assay, Equine dermal fibroblasts, Plant extracts, Wound healing

Introduction

Wound healing is a complex and dynamic biological process involving inflammation, cell proliferation, extracellular matrix deposition, angiogenesis, and tissue remodeling. In equines, wound management poses particular challenges due to the species' tendency for exuberant granulation tissue formation, infection susceptibility, and delayed epithelialization, especially in distal limb injuries (Fatima *et al.*, 2024) [1]. As such, there is a growing interest in identifying novel, effective, and natural therapeutic agents to enhance the wound healing process and minimize complications in horses.

Horses are frequently found to be more prone to secondary complication of wound healing such as excessive granulation tissue (proud flesh) formation (Dedar *et al.*, 2020a; Harman *et al.*, 2021) [2, 3]. Horses are also sensitive for the development of allergic dermatitis, photodermatitis (Williams *et al.*, 2011) [4] and skin tumors (MacGillivray *et al.*, 2002; Smith, 2008) [5, 6].

Medicinal plants have historically played a crucial role in traditional medicine systems, offering bioactive phytochemicals with anti-inflammatory, antimicrobial, and regenerative properties (Rates, 2001; Bhatt *et al.*, 2019) [8, 7]. Two such plants *A. tenuifolius* and *L. pyrotechnica* are of particular ethnomedicinal significance in South Asia and the Middle East.

A. tenuifolius, commonly known as wild onion, is traditionally used for its wound healing, antibacterial, and anti-inflammatory effects (potential). It contains a range of phytochemicals including flavonoids, saponins, and phenolic compounds, which are known to augment cell signaling and tissue regeneration (Bibi *et al.*, 2011) [9]. Recent *in vitro* studies have demonstrated its antioxidant and anti-proliferative activities, suggesting potential therapeutic applications. *L. pyrotechnica* found throughout the state of Rajasthan and seen in dry habitats particularly in desert zones. It is currently grown in forests, farm and road sides (Singh *et al.*, 2007; El-Fitany *et al.*, 2023) [11, 10]. It has been documented for its wound healing, anti-inflammatory, and immunomodulatory properties (El-Fitany *et al.*, 2023) [10]. Preliminary studies in animal models have shown improved wound contraction and collagen deposition following topical application of *L. pyrotechnica* extracts (Shaw *et al.*, 2014) [12].

Despite these promising attributes, the potential of these medicinal plants for wound management, particularly in equine cells, remains insufficiently explored. Plant-derived extracts may contain a diverse range of bioactive compounds with antioxidant, anti-inflammatory, antimicrobial, and tissue-protective properties that could contribute to wound healing. However, before their therapeutic application, it is essential to estab

lish their cellular safety and determine the concentrations that do not adversely affect cell viability. In vitro cytotoxicity assessment therefore provides an important preliminary step in evaluating the suitability of plant extracts for potential wound-healing applications. Accordingly, the objective of the present study was to evaluate and compare the in vitro cytotoxicity of polar and non-polar extracts of *Asphodelus tenuifolius* and *Leptadenia pyrotechnica* using the MTT assay. The findings may provide a scientific basis for assessing the safety and therapeutic potential of these plant extracts for the development of plant-derived approaches to equine wound management.

2. Materials and Methods

The present study was undertaken to comparatively evaluate the in vitro cytotoxicity of leaf extracts of *Asphodelus tenuifolius* and *Leptadenia pyrotechnica* using cultured equine dermal fibroblasts. Fresh leaves of plant were collected manually from Bikaner region. *Asphodelus tenuifolius* identified by Botany Department, Government Dungar College, Bikaner. *Leptadenia pyrotechnica* identified by Central Arid Zone Research Institute, Jodhpur. The collected leaves were thoroughly cleaned to remove adhering dust and foreign matter and were dried in a hot-air oven at 50 °C. The dried leaves were subsequently ground into a fine powder using a mixer grinder and stored appropriately until extraction.

Analytical-grade chemicals and reagents were used throughout the study. The experimental work involved the preparation of plant extracts, culture and maintenance of equine dermal fibroblast cells, treatment of cells with different concentrations of the plant extracts, and assessment of cell viability using the MTT assay.

2.1 Revival and cultivation of equine fibroblast cells

The current study involves the utilization of cryopreserved equine dermal fibroblast cells (third generation), which were previously stored at EPC-NRCE in Bikaner. In order to revive and facilitate the cultivation of these fibroblast cells, the cryopreserved cells were extracted from the liquid nitrogen cryocanister and immediately subjected to a 37 °C water bath for thawing purposes. The cryogenic containers were gently agitated until all ice crystals had dissipated. The contents of each cryovial were then transferred to 15 ml centrifuge tubes containing cell culture medium, and subsequently underwent two rounds of centrifugation for 3 min at 1000 rpm to generate a cell pellet. The supernatant was subsequently discarded and replaced with fresh medium. Following another round of centrifugation for 3 min at 1000 rpm and subsequent removal of the supernatant, the resulting cell pellet was suspended in 6 ml of culture medium. This suspension was then gently centrifuged, and 1 ml was extracted for cell counting

purposes. The remaining cell suspensions were then transferred to culture flasks and placed in an incubator containing 5% CO₂ and maintained at a temperature of 37 °C.

Preparation of petroleum ether extract

Exactly 50 gm of powder of each plant was taken in a glass bottle of 1000 ml capacity. Added petroleum ether (PE), to fill the bottle. It was kept at room temperature for 5 days. PE extract of *A. tenuifolius* and *L. pyrotechnica* having concentration of 1 mg/ml was diluted with ethanol 1000 times to make concentration 100 µg/ml in first well. Further 2-fold serial dilutions in ethanol were 100 µg/ml to 0.045 µg/ml made till 8th well.

2. Preparation of methanol ether extract

The remaining unfiltered sediment of petroleum ether extract is dried by a rotary evaporator to make powder. Exactly 50 gm of powder was taken in a glass bottle of 1000 ml capacity. Added methanol, to fill the bottle. It was kept at room temperature for 5 days. Methanolic extract of *A. tenuifolius* and *L. pyrotechnica* 1 mg/ml in ethanol was diluted in ethanol 1000 times to make 100 µg/ml to 0.045 µg/ml concentration of 100 µg/ml in first well. Further 2-fold serial dilutions in ethanol were made till 8th well.

3. Preparation of aqueous ether extract

The remaining unfiltered sediment of methanolic extract is dried with a rotary evaporator to make powder. Exactly 50 gm of powder was taken in a glass bottle of 1000 ml capacity. Added water, to fill the bottle. It was kept at room temperature for 5 days. Aqueous extract of *A. tenuifolius* and *L. pyrotechnica* having concentration of 1 mg/ml of ethanol was diluted with ethanol 1000 times to make concentration 100 µg/ml in first well. Further 2-fold serial dilutions in ethanol were 100 µg/ml to 0.045 µg/ml made till 8th well.

2. Cytotoxicity analysis

Cytotoxicity MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) was used (Nirwana *et al.*, 2021). Equine dermal fibroblasts maintained at EPC-NRCE, Bikaner, were trypsinised, seeded into culture plates, and allowed to attach. Cells were then treated with different concentrations of petroleum ether, methanolic, and aqueous extracts of *A. tenuifolius* and *L. pyrotechnica*. Following incubation, cell viability was determined by the MTT assay and expressed as a percentage of the untreated control. Extract concentrations maintaining > 80% cell viability were considered non-cytotoxic. The percentage of viable cells was calculated using the following formula :

$$\% \text{ Living cells} = \frac{\text{OD treatment} - \text{OD media}}{\text{OD cell control} - \text{OD media}} \times 100$$

3.0 Results

The cytotoxicity of various extracts from *A. tenuifolius* and *L. pyrotechnica* was evaluated using the MTT assay. Briefly, dermal fibroblast cells were treated with 2-fold serial dilutions (ranging from 100 µg/ml to 0.457 µg/ml) of the extracts for 72 hours in a CO₂ incubator (at 5% CO₂ and 37 °C temperature), alongside a vehicle control or culture medium only. The results of the cytotoxicity study for all extracts are provided below.

3.1 Cytotoxicity assay for Petroleum ether extract of *A. tenuifolius*

50 grams of *A. tenuifolius* leaf powder were used to prepare the Petroleum ether extract. The MTT assay indicated significant cytotoxicity at concentrations of 100 µg/ml (~91%), 33.3 µg/ml (~78%), Therefore, a concentration of 1.23 µg/ml was considered non-cytotoxic for dermal fibroblast cells. 11.11 µg/ml (~28%), and 3.70 µg/ml (~5%). Below 1.23 µg/ml concentration the cell viability were comparable with control and vehicle control (Table 1).

Table 1. Cytotoxicity assay for Petroleum ether extract of *A. tenuifolius*

S.N.	Concentration of extract (µg/ml)	Treated (% cell viability)	Concentration of solvent control (%)	Solvent control (% cell viability)
1.	100	9.19	2	9.19
2.	33.33	21.48	1	15.06
3.	11.11	72.07	0.5	72.07
4.	3.70	95.34	0.25	87.47
5.	1.23	105.04	0.12	88.73
6.	0.41	103.04	0.062	84.65
7.	0.14	122.03	0.031	99.02
8.	0.05	110.48	0.015	94.69

3.2 Cytotoxicity assay for Methanolic extract of *A. tenuifolius* - Similarly, fifty grams of *A. tenuifolius* leaf powder were used to prepare the methanolic extract. Significant cytotoxicity was observed at concentrations of 100 µg/ml (~90%), 33.3 µg/ml (~85%), 11.11 µg/ml (~57%), 3.70 µg/ml (~29%), 1.23 µg/ml (~27%), and

0.41 µg/ml (~8%). Below 0.14 µg/ml concentration the cell viability were comparable with control and vehicle control (Table 2). Hence, a concentration of 0.14 µg/ml was deemed non-cytotoxic for dermal fibroblast cells.

Table 2. Cytotoxicity assay for Methanolic extract of *A. tenuifolius*

S.N.	Concentration of extract (µg/ml)	Treated (% cell viability)	Concentration of solvent control (%)	Solvent control (% cell viability)
1.	100	10.32	2	9.19
2.	33.33	14.33	1	15.06
3.	11.11	42.38	0.5	72.07
4.	3.70	70.57	0.25	87.47
5.	1.23	72.35	0.12	88.73
6.	0.41	91.25	0.062	84.65
7.	0.14	108.2	0.031	99.02
8.	0.05	99.27	0.015	94.69

3.3 Cytotoxicity study of Aqueous extract of *A. tenuifolius*: Fifty grams of *A. tenuifolius* leaf powder were used to prepare the aqueous extract. The Aqueous extract from *A. tenuifolius* exhibited significant cytotoxicity at various concentrations, i.e., 100 µg/ml (~89%), 33.3 µg/ml (~88%), 11.11 µg/ml (~46%), 3.70 µg/ml (~44%),

1.23 µg/ml (~26%), 0.41 µg/ml (~26%), 0.14 µg/ml (~29%), and 0.05 µg/ml (~28%). Surprisingly, the aqueous extract showed higher toxicity compared to the petroleum or methanolic extracts (Table 3). A concentration 0.05 µg/ml was considered non-cytotoxic for dermal fibroblast cells.

Table 3. Cytotoxicity assay for Aqueous extract of *A. tenuifolius*

S.N.	Concentration of extract (µg/ml)	Treated (% cell viability)	Concentration of solvent control (%)	Solvent control (% cell viability)
1.	100	10.2	2	9.19
2.	33.33	10.9	1	15.06
3.	11.11	53.34	0.5	65.67
4.	3.70	54.74	0.25	87.47
5.	1.23	73.20	0.12	88.73
6.	0.41	73.63	0.062	84.65
7.	0.14	70.52	0.031	99.02
8.	0.05	72.15	0.015	94.69

3.4 Cytotoxicity study of Petroleum ether extract of *Leptadenia pyrotechnica* : 50 grams of *L. pyrotechnica* leaf powder was utilized to prepare the Petroleum ether extract. Significant cytotoxicity was observed at concentrations of 100 µg/ml (~90%) and 33.3 µg/ml

(~89%). Below 3.70 µg/ml concentration the cell viability were comparable with control and vehicle control (Table 4). Hence, a concentration of 3.70 µg/ml was considered non-cytotoxic for dermal fibroblast cells.

Table 4. Cytotoxicity assay for Petroleum ether extract of *L. pyrotechnica*

S.N.	Concentration of extract (µg/ml)	Treated (% cell viability)	Concentration of solvent control (%)	Solvent control (% cell viability)
1.	100	9.68	2	9.19
2.	33.33	10.16	1	15.06
3.	11.11	99.26	0.5	65.67
4.	3.70	100.3	0.25	87.47
5.	1.23	103.2	0.12	88.73
6.	0.41	101.9	0.062	84.65
7.	0.14	99.43	0.031	99.02
8.	0.05	103.75	0.015	94.69

3.5 Cytotoxicity study of Methanolic extract *Leptadenia pyrotechnica*: Similarly, using fifty grams of *L. pyrotechnica* leaf powder, the methanolic extract was prepared. Significant cytotoxicity was observed at concentrations of 100 µg/ml (~91%), 33.3 µg/ml (~88%), 11.11 µg/ml (~13%), 3.70 µg/ml (~9%), 1.23 µg/ml (~9%), and

0.41 µg/ml (~14%) (Table 5). However, below 0.05 µg/ml concentration the cell viability were comparable with control and vehicle control. Therefore, a concentration of 0.05 µg/ml was deemed non-cytotoxic for dermal fibroblast cells.

Table 5. Cytotoxicity assay for Methanolic extract of *L. pyrotechnica*

S.N.	Concentration of extract (µg/ml)	Treated (% cell viability)	Concentration of solvent control (%)	Solvent control (% cell viability)
1.	100	8.7	2	9.19
2.	33.33	11.85	1	15.06
3.	11.11	86.56	0.5	65.67
4.	3.70	90.91	0.25	87.47
5.	1.23	90.59	0.12	88.73
6.	0.41	92.86	0.062	84.65
7.	0.14	85.53	0.031	99.02
8.	0.05	105.89	0.015	94.69

3.6 Cytotoxicity study of Aqueous extract *Leptadenia pyrotechnica*: Using fifty grams of *L. pyrotechnica* leaf powder, the aqueous extract was prepared. The Aqueous extract from *L. pyrotechnica* demonstrated significant cytotoxicity at various concentrations, i.e.,

100 µg/ml (~91%), 33.3 µg/ml (~84%), 11.11 µg/ml (~28%), 3.70 µg/ml (~14%), 1.23 µg/ml (~8%), 0.41 µg/ml (~9%), and 0.14 µg/ml (~14%) (Table 6). A concentration of 0.05 µg/ml was identified as non-cytotoxic for dermal fibroblast cells.

Table 6. Cytotoxicity assay for Aqueous extract of *L. pyrotechnica*

S.N.	Concentration of extract (µg/ml)	Treated (% cell viability)	Concentration of solvent control (%)	Solvent control (% cell viability)
1.	100	9.07	2	9.19
2.	33.33	16.98	1	15.06
3.	11.11	72.01	0.5	65.67
4.	3.70	85.78	0.25	87.47
5.	1.23	92.27	0.12	88.73
6.	0.41	91.35	0.062	84.65
7.	0.14	86.92	0.031	99.02
8.	0.05	101.89	0.015	94.69

% cell viability

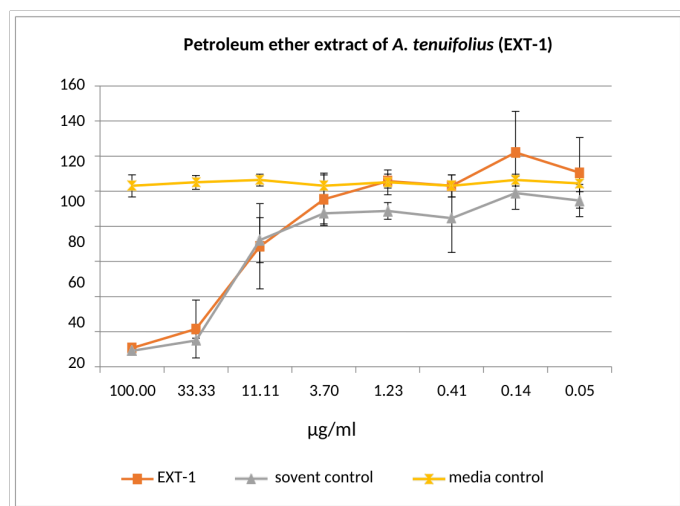


Figure 2. Cytotoxicity assay for Methanolic extract of *A. tenuifolius*

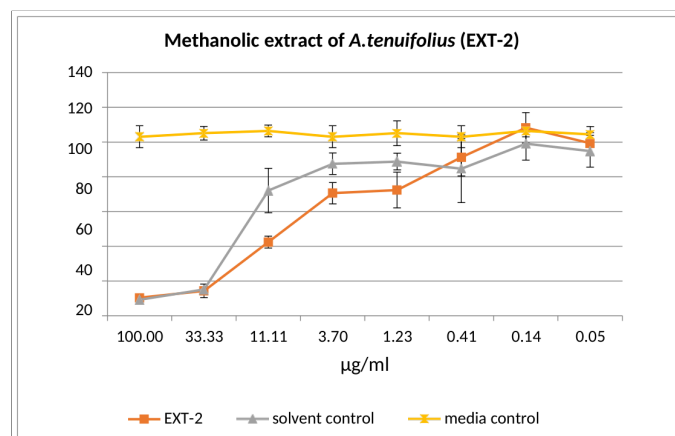


Figure 1. Cytotoxicity assay for Petroleum ether extract of *A. tenuifolius*

% cell Viability
% cell viability

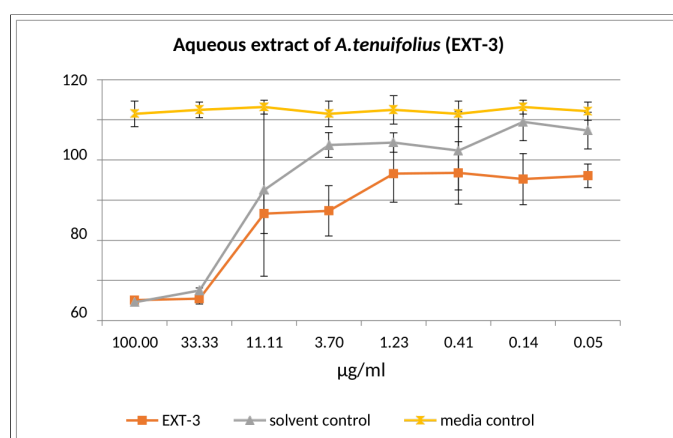
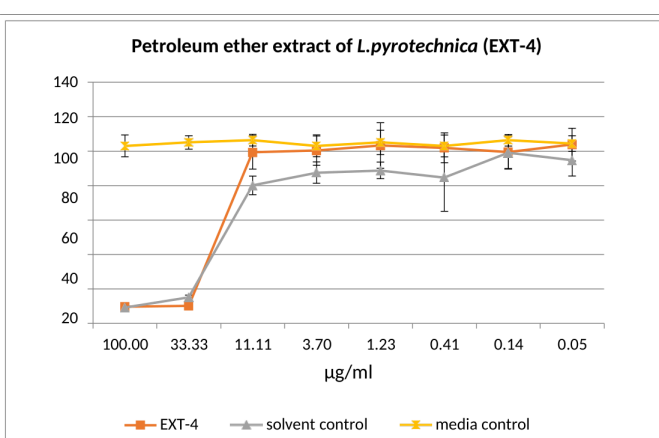


Figure 3. Cytotoxicity assay for Aqueous ether extract of *A. tenuifolius*



% cell Viability

Figure 4. Cytotoxicity assay for Petroleum ether extract of *L. pyrotechnica*

% cell viability

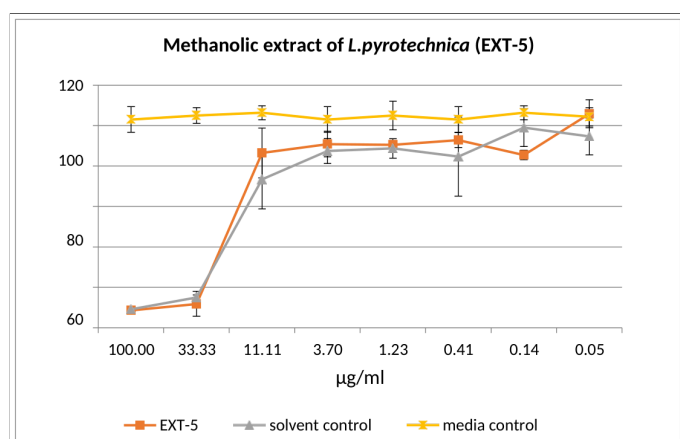


Figure 5. Cytotoxicity assay for methanolic extract of *L. pyrotechnica*

% cell viability

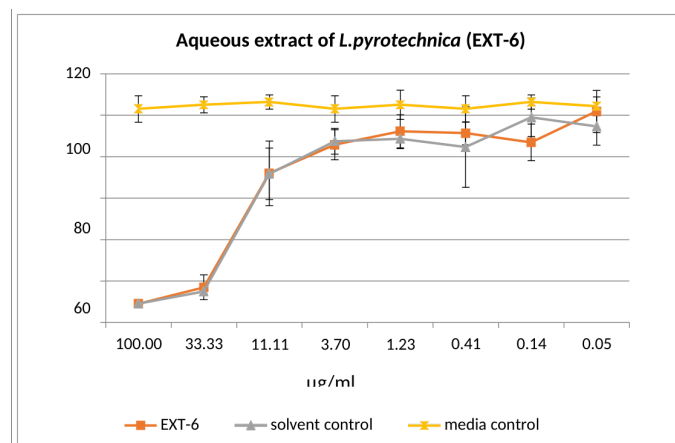


Figure 6. Cytotoxicity assay for aqueous extract of *L. pyrotechnica*

4. Discussion

Wound healing is a complex and highly coordinated biological process involving inflammation, proliferation, tissue formation and remodelling. Dermal fibroblasts are important cells in this process because they contribute to extracellular matrix formation, collagen synthesis and restoration of connective tissue during wound repair. Therefore, *in vitro* fibroblast-based models are useful for preliminary evaluation of the cellular compatibility and therapeutic potential of plant-derived extracts intended for wound management (Eming *et al.*, 2014; You and Han, 2014) [13, 14]. In the present study, the cytotoxicity of petroleum ether, methanolic and aqueous leaf extracts of *A.*

tenuifolius and *L. pyrotechnica* was evaluated in equine dermal fibroblasts using the MTT assay. Cytotoxicity assessment was performed before subsequent biological investigations to identify concentrations that were well tolerated by the cells. The extracts were tested over a wide concentration range from 0.05 to 100 µg/ml. The results demonstrated clear differences in cellular viability depending on the plant species, extraction solvent and extract concentration.

The petroleum ether extract of *A. tenuifolius* showed pronounced cytotoxicity at the higher concentrations tested. Cell viability was 9.19% at 100 µg/ml and 21.48% at 33.33 µg/ml, whereas a progressive increase in viability was observed with decreasing extract concentration. At 3.70 µg/ml, 1.23 µg/ml, 0.41 µg/ml, 0.14 µg/ml and 0.05 µg/ml, cell viability increased to 95.34%, 105.04%, 103.04%, 122.03% and 110.48%, respectively. These findings indicate that the cytotoxic effect of the petroleum ether extract was strongly concentration-dependent, with substantially better cellular compatibility at lower concentrations.

The methanolic extract of *A. tenuifolius* exhibited a similar concentration-dependent response. Cell viability was markedly reduced at 100 µg/ml (10.32%) and 33.33 µg/ml (14.33%), followed by progressive recovery at lower concentrations. At 11.11 µg/ml, viability increased to 42.38%, while concentrations of 3.70 µg/ml and below resulted in considerably higher viability. At the lowest concentration tested (0.05 µg/ml), cell viability reached 99.27%. Thus, although the methanolic extract exhibited substantial cytotoxicity at higher concentrations, it was considerably better tolerated by equine dermal fibroblasts at lower concentrations.

The aqueous extract of *A. tenuifolius* also showed a concentration-related reduction in cell viability at higher concentrations. Cell viability was 10.20% at 100 µg/ml and 10.90% at 33.33 µg/ml. However, viability increased at lower concentrations, reaching 73.20%, 73.63%, 70.52% and 72.15% at 1.23, 0.41, 0.14 and 0.05 µg/ml, respectively. Compared with the petroleum ether and methanolic extracts, the aqueous extract showed a somewhat different response pattern, suggesting that the biological effects of *A. tenuifolius* are influenced by the polarity and chemical composition of the extraction fraction.

A comparatively different cytotoxicity profile was observed for *L. pyrotechnica*. The petroleum ether extract showed marked cytotoxicity at 100 µg/ml and 33.33 µg/ml, with cell viabilities of 9.68% and 10.16%, respectively. However, viability increased sharply at lower concentrations, reaching 99.26% at 11.11 µg/ml and remaining above 99% at several subsequent concentrations. At 0.05 µg/ml, cell viability was 103.75%. This indicates that the petroleum ether extract of *L. pyrotechnica* was highly cytotoxic at the highest concentrations but showed good cellular compatibility at lower concentrations.

The methanolic extract of *L. pyrotechnica* demonstrated relatively favourable cytocompatibility at concentrations below 11.11 µg/ml. Cell viability increased from 8.70% at 100 µg/ml to 86.56% at 11.11 µg/ml and 90.91% at 3.70 µg/ml. At 1.23, 0.41, 0.14 and 0.05 µg/ml, viability was 90.59%, 92.86%, 85.53% and 105.89%, respectively. These findings suggest that the methanolic extract was comparatively well tolerated by equine dermal fibroblasts within the lower concentration range.

Similarly, the aqueous extract of *L. pyrotechnica* exhibited a concentration-dependent improvement in cell viability. At 100 µg/ml, cell viability was only 9.07%, whereas it increased to 72.01% at 11.11 µg/ml and 85.78% at 3.70 µg/ml. At lower concentrations, viability remained relatively high, reaching 92.27%, 91.35%, 86.92% and 101.89% at 1.23, 0.41, 0.14 and 0.05 µg/ml, respectively. Overall, the aqueous extract demonstrated good cellular compatibility at the lower concentrations evaluated.

The concentration-dependent cytotoxicity observed in the present study may be attributed to differences in the type and relative abundance of biologically active phytochemicals present in the extracts. Different extraction solvents selectively recover phytoconstituents according to their polarity and physicochemical characteristics, thereby producing extracts with distinct chemical profiles and biological activities. Previous investigations have demonstrated that solvent selec-

tion can substantially influence the phenolic and flavonoid composition and, consequently, the biological properties of plant extracts (Khasawneh *et al.*, 2011; Benariba *et al.*, 2021) [16, 15]. Therefore, cytotoxicity observed with a crude plant extract should not necessarily be regarded as a fixed characteristic of the plant species itself, but rather as a response influenced by extraction solvent, phytochemical composition and concentration.

Asphodelus tenuifolius Cav., belonging to the family Asphodelaceae, is an annual medicinal herb traditionally used for several therapeutic purposes, including inflammatory, rheumatic, digestive and cardiometabolic conditions. Various pharmacological investigations have reported antibacterial, antifungal, anti-inflammatory, antioxidant, diuretic and other biological activities associated with this plant (Mahmood *et al.*, 2011; Laouini *et al.*, 2015; Aslam *et al.*, 2016; Ouhaddou *et al.*, 2020) [18, 19, 17, 20]. More recent phytochemical investigations using chromatographic and mass-spectrometric approaches have further demonstrated the presence of diverse phenolic and flavonoid constituents in *A. tenuifolius* extracts, supporting the considerable chemical diversity of this species (Benariba *et al.*, 2021) [15]. Such phytochemical diversity may contribute to the biological responses observed in the present cytotoxicity study.

Previous phytochemical studies of *A. tenuifolius* have reported phenolic compounds, flavonoids, tannins, alkaloids, steroids and other secondary metabolites in different extracts. Compounds such as caffeic acid, chlorogenic acid, rutin, apigenin, luteolin and related polyphenolic constituents have been reported from the plant (Laouini *et al.*, 2015; Benariba *et al.*, 2021) [19, 15]. The biological effects of these constituents may vary according to their concentration and combination within a particular extract. Phenolic compounds and flavonoids are well known for their antioxidant, anti-inflammatory and other pharmacological activities; however, at sufficiently high concentrations, some phytochemicals may interfere with cellular metabolic processes and adversely affect cell viability. Thus, the concentration-dependent reduction in cell viability observed in the present study may reflect the cumulative effects of multiple constituents rather than the action of a single phytochemical.

The cytotoxic response of *A. tenuifolius* extracts may therefore be explained by differences in phytochemical composition among the petroleum ether, methanolic and aqueous fractions. Non-polar solvents are more likely to extract lipophilic constituents, whereas methanol and water preferentially recover relatively polar compounds, including phenolic acids, flavonoids and other hydrophilic metabolites. Consequently, differences in cellular response among the three extracts may reflect differences in the quantity and composition of the compounds extracted. Similar solvent-dependent variation in the phytochemical profile and biological activity of *A. tenuifolius* has been reported previously (Benariba *et al.*, 2021) [15].

Leptadenia pyrotechnica (Forssk.) Decne is a medicinal plant traditionally associated with several therapeutic applications. Older literature places the species within Asclepiadaceae, whereas current taxonomic treatments generally include it within Apocynaceae. The plant has been investigated for antioxidant, anti-inflammatory, antimicrobial and other pharmacological activities. Its biological properties have been associated with a chemically diverse range of secondary metabolites, including phenolic acids, flavonoids, alkaloids, terpenoids, sterols and glycosidic constituents (Khasawneh *et al.*, 2011; Javid *et al.*, 2019; Alqahtani *et al.*, 2022) [16, 22, 21].

Importantly, Khasawneh *et al.* (2011) [16] demonstrated considerable variation in the polyphenolic composition of different fractions of *L. pyrotechnica*. Gallic acid, vanillic acid, caffeic acid, epicatechin and quercetin-3-β-D-glucoside were identified in the extracts, with substantial differences in their concentrations among ethanol, ethyl acetate, n-butanol and aqueous fractions. The ethyl acetate fraction contained particularly high levels of total phenolics and showed pronounced biological activity, demonstrating that the pharmacological properties of *L. pyrotechnica* can vary according to the fractionation procedure and chemical composition (Khasawneh *et al.*, 2011) [16]. More recent phytochemical studies have also confirmed the presence

of caffeic, gallic, vanillic, ferulic, *p*-coumaric and cinnamic acids, together with flavonoid and terpenoid constituents in different extracts of the plant (Alqahtani *et al.*, 2022; Al-Salman *et al.*, 2024) [21, 23].

The presence of these phytochemicals may partly explain the concentration-dependent cellular response observed in the present study. Phenolic acids and flavonoids can exert beneficial biological effects at suitable concentrations, whereas increasing concentrations may alter cellular redox status, membrane integrity or metabolic activity. The observed reduction in cell viability at higher extract concentrations is therefore consistent with the possibility that increasing concentrations result in greater cellular exposure to biologically active constituents. However, because the present study evaluated crude extracts rather than individual purified compounds, the contribution of specific phytochemicals to cytotoxicity cannot be established directly. When the two plant species were compared, *L. pyrotechnica* generally demonstrated better cellular compatibility at several of the lower concentrations tested, particularly in its methanolic and aqueous extracts. In contrast, *A. tenuifolius*, particularly its petroleum ether and methanolic extracts, showed comparatively greater cytotoxicity at the highest concentrations. Nevertheless, both plant species exhibited substantial recovery of cell viability following reduction in extract concentration. These findings indicate that cytocompatibility was influenced not only by plant species but also by extraction solvent and concentration. Such solvent-dependent differences are consistent with previous studies demonstrating that different fractions of medicinal plants can possess markedly different phytochemical compositions and biological activities (Khasawneh *et al.*, 2011; Benariba *et al.*, 2021) [16, 15].

The MTT assay provides an indirect measure of cellular viability and metabolic activity through the reduction of the tetrazolium salt MTT to a coloured formazan product by metabolically active cells (Mosmann, 1983) [24]. Therefore, the marked decrease in MTT-derived viability at higher extract concentrations indicates reduced cellular metabolic activity under those conditions. However, because the MTT assay reflects cellular metabolic activity rather than viability alone, the results should be interpreted as an indicator of metabolic cytotoxicity or reduced cellular activity. Additional assays assessing membrane integrity, apoptosis or oxidative stress would be useful to confirm the mechanism underlying the observed reduction in viability.

From the perspective of equine wound management, cytocompatibility is an important preliminary criterion for evaluating plant-derived candidates for topical wound-healing applications. Fibroblasts play a central role in extracellular matrix deposition, collagen synthesis, tissue remodelling and wound contraction. Therefore, an extract intended for further wound-healing evaluation should ideally maintain adequate fibroblast viability at concentrations that are capable of producing beneficial biological effects. The present findings indicate that both *A. tenuifolius* and *L. pyrotechnica* contain extract fractions that can be tolerated by equine dermal fibroblasts when applied at appropriately selected concentrations. This provides a preliminary basis for subsequent investigation of their effects on wound-healing-associated cellular responses.

The comparatively favourable cytocompatibility of *L. pyrotechnica* at lower concentrations is noteworthy because previous investigations have demonstrated considerable phenolic diversity in this plant and have identified compounds such as epicatechin, vanillic acid, caffeic acid and quercetin derivatives (Khasawneh *et al.*, 2011) [16]. Similarly, the phytochemical diversity of *A. tenuifolius* may account for the biological activity observed in its different solvent extracts (Laouini *et al.*, 2015; Benariba *et al.*, 2021) [19, 15]. Nevertheless, the relationship between individual phytochemicals and fibroblast viability requires further investigation using chemical profiling and purified compounds.

Overall, the present cytotoxicity study demonstrated a clear concentration-dependent response to petroleum ether, methanolic and aqueous extracts of both *A. tenuifolius* and *L. pyrotechnica*. Higher concentrations generally produced greater reductions in cell viability,

whereas lower concentrations were comparatively better tolerated. Among the extracts evaluated, *L. pyrotechnica* generally showed comparatively favourable cytocompatibility at lower concentrations. The differences observed between extracts may be associated with variation in phytochemical composition resulting from both plant species and extraction solvent. These findings support the selection of appropriately tolerated, non-cytotoxic concentrations for subsequent evaluation of the wound-healing potential of these medicinal plants in equine dermal fibroblasts. Further studies integrating phytochemical profiling with mechanistic cellular assays would be valuable for identifying the constituents responsible for the observed biological effects.

5. Conclusion

The extracts of *Asphodelus tenuifolius* and *Leptadenia pyrotechnica* showed concentration-dependent cytotoxicity in equine dermal fibroblasts. Lower concentrations were generally well tolerated, with *L. pyrotechnica* extracts demonstrating comparatively favourable cytocompatibility. These findings provide a basis for selecting safe concentrations for further evaluation of their potential in equine wound healing.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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