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Cell viability assay for aqueous and ethanolic extract of *Capparis deciduas*

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Abstract
The present study was taken to investigate cell viability assay for aqueous and ethanolic extract of *Capparis decidua*. Skin tissue was collected from horse and processed via sequential enzymatic tissue digestion and tissue explant method. Dermal fibroblast cells were maintained in DMEM culture medium supplemented with 10% FBS in a humidified incubator at 37 °C and 5 % CO_2 level. Aqueous and ethanolic extracts of these herbs were made through soxhlet apparatus and evaoporated by rotatory evaporative to get dried extract. MTT assay was used to evaluate noncytotoxic concentration of aqueous and ethanolic extracts of *Capparis decidua.* Aqueous extract of *Capparis deciduas* have shown more than 80% cell viability in all dilution concentrations. Ethanolic extract of *Capparis* has showed cytotoxicity up to 8 µg/ml concentration. So, it is concluded that *Capparis deciduas* have great therapeutic potential to treat variety of diseases because of active phytochemical that present abundantly in it.

Keywords: Capparis decidua, aqueous extract, ethanolic extract, cell culture, MTT assay

1. Introduction
Recently, medicinal plants have gained increasing popularity due to their phytoconstituents, which have been shown to have medicinal properties. Among these plants is *Capparis decidua*, widely used in traditional medicine. The perennial shrub *Capparis deciduas*, commonly referred to as Kair, is commonly found in dry and arid regions of Rajasthan, Gujarat, Punjab and Western Ghats of India. As a result, it has great economic importance in the Mediterranean area because of the edible buds and fruits (caper berries), which are usually consumed as pickles (Talili *et al*., 2011). In different parts of the plant, phytosterols, tocopherols, carotenoids, flavonoids, glucosinolates are found. However, there are several major flavonoids in fruit and leaves, including kampferol, quarcetin, sitosterol, styderine, isorhamnetin and their O-methyl derivatives, thomnocitirin, rhamnetin and rhamnozin. Some of these isolated compounds have medicinal uses such as antioxidative, antifibrotic, antifungal, antihepatotoxic, anti-inflammatory, anti-dibetic, antihypertensive, antihyperlipidemic, antibacterial, antiparasital, immunostimulant and antitumor activities (Attar *et al*., 2021) [3] Aqueous and ethanolic extract of *Capparis deciduas* have cytoprotective effect to normal fibroblast cells due to flavinoids and phenolic compounds. So, *Capparis deciduas* found to be have great therapeutic potential to treating variety of disease.

2. Materials and Methods
The present work has been carried out at Equine Production Campus, ICAR-NRC on Equines, Bikaner, Rajasthan. Present research work was approved by the institute animal ethics committee of college of veterinary and animal science Bikaner. All the chemicals were purchased from Sigma (USA) and the plastic ware from Eppendorf (Roskilde, Denmark) unless and until mentioned otherwise.

2.1 Fibroblast cell isolation and culture
Dermal Fibroblast Cell isolation and culture was done as previously described method by (Pollard and walker, 1997) [5]. In brief, the collected skin sample from neck or beneath the tail region of horse in steril conditions and were washed with normal saline (0.9%) three to four times. After washing, tissue was cut in small pieces with a surgical blade and scissors and digest with 2% trypsin for 1 hour at 37 ºC. The digested samples were washed in phosphate-buffered saline and centrifuged at 2500 RPM for 10 min. The cell pellet was resuspended in basal culture medium composed of low glucose Dulbecco's modified Eagle's medium

(DMEM) containing 10% foetal bovine serum (FBS). The cells were cultured in a humidified atmosphere with 5% CO2 at 37.5 ºC. The basal culture medium was changed two times a week and passaged after reaching 80 % confluency.

2.2 Crypreservation of dermal fibroblast
Dermal fibroblast Cells were cryopreserved after passage. Briefly described, the fibroblast cells in the flask were initially washed two times with DPBS and EDTA-trypsin was added to remove the adherent cells. The flask was treated for 3 minute at 37 ºC and observed under the microscope for the cell removal from the surface. After ensuring the cell removal, 4 ml of DPBS was added and the contents were emptied in a 15 ml tube and centrifuged for 1000 RPM for 5 minutes. Meanwhile, the cryopreservation media using Dimethyl Sulfoxide (DMSO) (10% of the culture media) was prepared and kept ready. The cell pellet obtained after removal of the supernatant was mixed with 1 ml of cryopreservation media (DMSO and Culture media) and were initially stored on 4 ºC, -20 ºC, then -80ºC and shifted to LN2 storage cryocans.
Cryovials were thawed in a 37 °C water bath for 2 minutes till the liquid portion appears in the cryovial. The cells were immediately transferred to 5 ml of culture media and gently vortexed. The cells were centrifuged at 1000 RPM for 4 minutes at room temperature. The cell pellet was resuspended in the culture media. The cell counts were made for the effect of cryopreservation in the cell viability.

2.3 Plant Material
About 1 Kg of fruit of *Capparis decidua* was collected from local area of Bikaner and were positively identified with the authentic sources.

2.4 Drying & size reduction
The freshly collected *Capparis decidua* were shade dried under normal environmental conditions and then make to coarse powder.

2.5 Preparation of extract
The fresh fruit of *Capparis deciduas* were collected and shade dried. The dried material was reduced to coarse powder in a mechanical grinder to obtain about powder of desired particle size. Powdered material was subjected to successive extraction with aqueous and ethanol. For this powdered material soaked in aqueous and ethanol in equal amount respectively for 5 days. After that Sonicate the material using sonicator (model VCX 750, Made sonics & Material, USA) at max. temp of 60^0 C, pulse for 59 seconds and amplitude of 70% under ice for 20 min. Sonicated extracts were filtered using whatman filter paper no.1 and dried through rotary evaporation. Prepared aqueous and ethanolic extracts were kept in the refrigerator at 4 to 5 ºC for further use within a month.

2.6 Cell viability assay
To study cytotoxicity MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) was used (Nirwana *et al.*, 2011) [6]. Different concentrations of dilution of aqueous and ethanolic extracts of herbs *Capparis deciduas* were put into a 96 well microplate containing 50 µl Dulbecco eagle's minimum essential medium (DMEM) culture media and 50µl dermal fibroblasts suspension with one media control group, and one cell control group. For the ethanolic extract, wells having equal dilutions of ethanol in media were used as control solvent. The microplate was incubated in a 37 °C CO_2 incubator for 72 h. The cell growth media were then removed and washed with 100 µl PBS solution and repeated twice. MTT @ 0.5 mg/ml concentration was added to every well and incubated at 37 ºC for 4 hour. For each well, 50 µl of dimethyl sulfoxide (DMSO) was added. At 24 and 72 h after incubation, the microplate was shaken using a plate shaker for 5 min until the formazan crystals had dissolved. Fibroblast living cells were colored with formazan purplish blue, as the dead cells do not turn the purplish blue color. The formazan absorbance was read using an ELISA reader with a wavelength of 540 nm. OD of each well was read and expressed as percentage cell survival (absorbance of treated wells/absorbance of control wells ×100). From the result it can be interprete that activity of plant extract was significant which gave less than 50% survival at exposure time of 48 h. Then extract diluted and make 8 concentation by making 3 fold serial dilutions of extract. Each concentration applied in the wells in triplet and incubated for further 48 h. Its cytotoxic effect was determined by MTT assay.

3. Results
Results are presented in table 1 and table 2:

Table 1: MTT assay for aqueous extract of *C. decidua* (kair)

S. No.	Concentrations (µg/ml)	Treated (Percent cell viability)
1.	148.00	87.40
2.	49.33	89.20
3.	16.44	107.00
4.	5.48	87.27
5.	1.83	87.33
6.	0.61	90.10
7.	0.20	112.90
8.	0.07	86.87

Table 2: MTT assay for ethanolic extract of *C. decidua* (kair)

S. No.	Concentrations (µg/ml)	Treated (Percent cell viability)	Solvent Control (Percent cell viability)
1.	220.00	9.07	11.37
2.	73.33	50.67	56.47
3.	24.44	72.37	71.47
4.	8.15	72.83	80.47
5.	2.72	85.93	81.43
6.	0.91	67.20	106.40
7.	0.30	100.83	110.70
9.	0.10	92.43	90.63

4. Discussion
By using equine dermal fibroblast cells, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was used to study cytotoxicity. Using all concentrations in the study, the aqueous extract of *Capparis deciduas* showed over 80% cell survival (Table 1), which indicates its therapeutic and nutritional potential. The ethanolic extract of *Capparis deciduas* was, however, cytotoxic up to a concentration of 8 µg/ml (Table 2). It has been shown that Capparis decidua contains simiarenol, stydrine, ksmpherol, lupeol, β-sitosterol triacontenate and taraxerol in its ethanol extract (Rathee *et al*., 2012a; Anjum *et al*., 2020) [8, 2]. Seeds contain glucocapparin, an antibacterial principle, and isothiocyanate aglycone of glucocapparin. Active phychemicals present in *Capparis* are responsible for its biological activity such as antifibrotic,

antitumor, cell proliferation and migration, wound healing, antioxidant and anti- apoptotic activity. It has been shown that extract of *Capparis deciduas* possess cytoprotective effect on normal fibroblast cells and prevent cell death but on application on cancerous cell lines it shows cytotoxic effect. It is because of active phytochemical of *Capparis*, beta sitosterol that is found to be cytotoxic to A549 cancer cells and to be a powerful antimetastatic agent, which can significantly inhibit malignant cell metastatic and invasive abilities. IC_{50} value of the β-Sitosterol triacontenate was found to be 1 µM. Moreover, it is reported that the extract increased wound healing rates by stimulating granulation tissue, epidermal regeneration, angiogenesis, collagen, TGF-b, and VEGF production (Okur *et al*., 2018) [7] due to high phenolic and flavinoid content (Rathee *et al*., 2012) [9]. There is evidence that the aqueous extract of *C. decidua* had higher cell protective activity than the methanolic extract, possibly because it contains more polar phytoconstituents (Ali *et al*., 2019) [1]. Polyphenolic flavonoids like quercetein, found in capparis, are powerful anti-inflammatory and antioxidants. As quercetein may play a role in inhibiting the progression of fibrosis through inhibiting the p38 MAPK signaling pathway (Wang *et al*., 2017) [14]. It is reported that *C. decidua* attenuating oxidative damage, cell arrest, prevent inflammation, and cell death (Santhakumar *et al*., 2021) [11]. According to results it is shown that ethanolic extract of capparis have cytotoxic effect up to 8 µg/ml concentration, on dilutions of extract it shows cytoprotective effect and helps in prolifection of cells. It may be because of flavinoids isolated from fruit. It is shown that at the concentration of 12.5 and 25 µg/ml, kampferol significantly suppress the release on anti inflammatory and profibroticcytokines, including IL-6, IL-1β, IL-18, TGF-β and TNF-α in sclerotic skin (Tang *et al*., 2015) [12]. It is also demonstrated that kaempferol remarkably attenuated collagen synthesis, proliferation and activation of fibroblasts *in vitro* and *in vivo*. Such bioactivity of kaempferol which resulted from the inhibition of TGF-β1/Smads signaling. So it is promising agent for the treatment of fibroproliferative disorders (Li *et al*., 2016) [4]. It also have cytoprotectiv effect beause of reducing oxidative stress, inflammation, and oxidative cellular damage (Sekiguchi *et al*., 2019) [10].

5. Conclusion

So it can be concluded that capparis decidua aqueous extracts has shown least cytotoxicity and prevent cell damage. So there is possibilities for clinical use of these herbs as analgesic, antifibrotic, anti-inflammatory, skin repair, wound healing and anti-tumor agents. Further studies are warranted to test the efficacy of the herbal extracts on large number of clinical cases *in-vivo* to strengthen this concept.

6. Conflicts of interests

There was no conflict of interests.

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