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Research Article

Evaluation of Wound Healing Effect of *Dodonaea viscosa* Linn. by Cell Proliferation Assay

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ABSTRACT
The medicinal plant *Dodonaea viscosa* (Sapindaceae) has been used traditionally in the treatment of various diseases such as malaria, ulcers, dysmenorrhoea, rheumatism, sprains, bruises, burns and wounds. This study was aimed to investigate the effect of ethanol extract and flavonoid rich fraction of *Dodonaea viscosa* on a simplified *in vitro* wound healing study. Cultured Keratinocytes (HACAT) were exposed to ethanol extract and flavonoid rich fraction at different concentrations for 48 hours. The resultant cellular proliferation was determined after 48 hours by MTT assay and calculated relatively to control. Flavonoid rich fraction of the *Dodonaea viscosa* induced a significant cell proliferation after 48 hours exposure, when compared to the control group. The flavonoids rich fraction of the *Dodonaea viscosa* has better efficiency in inducing cell proliferation than ethanol extract. The cell proliferation assay can be used as a promising scientific approach and platform to evaluate plant extract known for their wound healing property.

Key words: *Dodonaea viscosa*, Wound healing, Flavonoids.

INTRODUCTION

Dodonaea viscosa Linn. (Sapindaceae) is an evergreen shrub distributed throughout in India[1]. This plant is commonly known as Hop bush plant in English and Sinatha in Hindi[2,3]. The stem, leaves, seeds, roots, bark and aerial parts are used in traditional medicine. Traditionally the leaves are used in the treatment of fever, malaria, ulcers, diarrhea, dysmenorrhoea, rheumatism, sprains, bruises, burns and wounds[4-6]. It is proved to have antibacterial, antiviral, analgesic, anti-inflammatory, antiulcer and antioxidant activity[7-9]. Literature showed the presence of flavonoids, diterpenoid acids, saponins, *P*-coumarin acid ester, sterols, essential oils and tannins[10-13]. Wound healing is a complex process encompasses migration to the wounded area, proliferation, deposition and remodeling of extracellular matrix[14]. A variety of materials for wound dressing, skin substitutes and recombinant growth factors which are shown to enhance the healing process entered into the market with therapeutic efficacy[15]. Many natural products and extracts are also known to possess wound healing properties[16-18]. Hence, there is a great interest to find new wound healing products. Based on the folklore claim of *Dodonaea viscosa*, this study was designed to explore the wound healing potential of this plant. We used a Cell proliferation assay with human keratinocytes which is a well-established wound healing model [19-21].

MATERIALS AND METHODS

Plant collection and authentication

The fresh healthy plant leaves of *Dodonaea vicosa* Linn. were collected from the Alagarkovil Hills, Madurai, TamilNadu, India during the month of August 2010. The plant was authenticated by Dr. P. Jayaraman, Botanist, Plant anatomical research centre, Chennai and a voucher specimen has been deposited in the Department of Pharmacognosy.

Preparation of ethanol extract

Coarse powder was defatted with Petroleum ether and the marc was macerated with ethanol for 72 hours. The extract was filtered and concentrated in rotary flash evaporator.

Preparation of flavonoid rich fraction

The ethanol extract was suspended in distilled water and

Table 1: Effect of Flavonoids rich fraction and Ethanol extract of *Dodonaea viscosa* on Cell proliferation assay

Sl.no	Concentrations (µg/ml)	Percentage growth	
		Flavonoids rich fraction	Ethanol extract
1	1	69±1.3	45±0.8
2	3	96±2.7	54.56±1.5
3	10	104±1.5	63.12±2.1
4	30	112±3.1	67.85±3.4
5	100	55±0.9	25±0.5

Data are expressed as mean±SD from three individual experiments.

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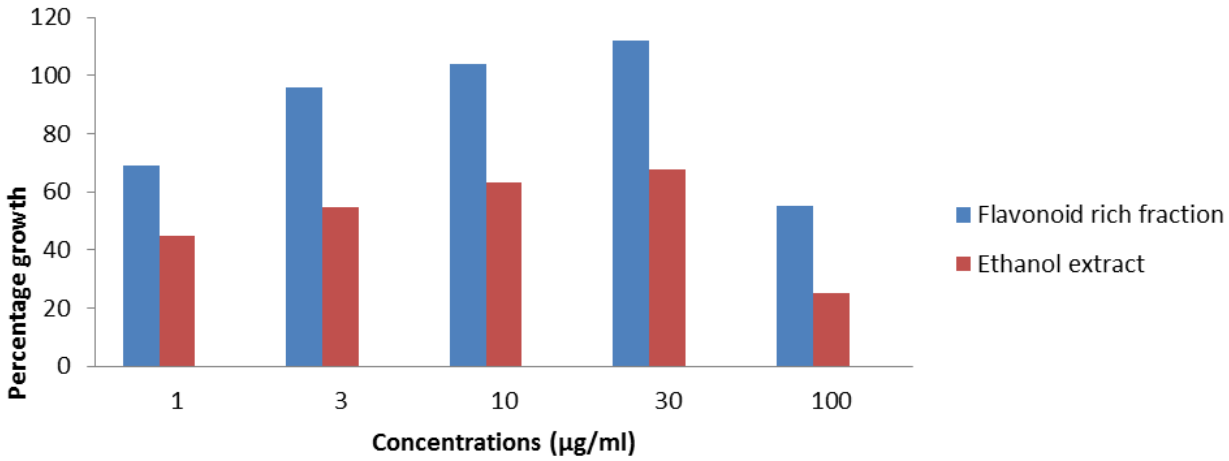

Fig.1: Effect of *Dodonaea viscosa* on Cell Proliferation assay

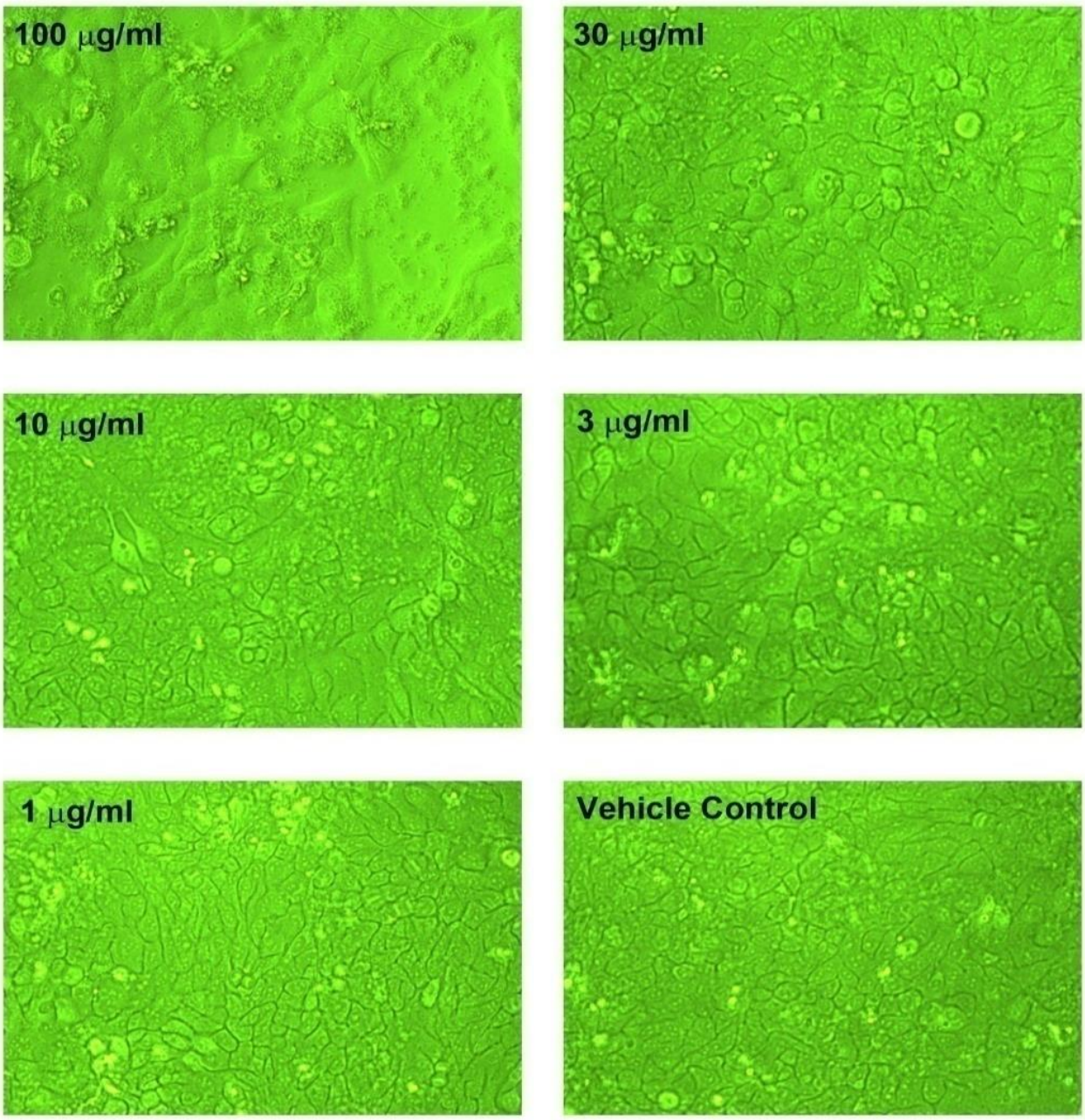

Fig.2: Fraction treated HaCaT Cells

then partitioned with n-Hexane, Ethyl acetate and n-Butanol successively. Each portion was evaporated under reduced pressure to yield Hexane, Ethyl acetate, Butanol and Aqueous fractions respectively. The ethyl acetate fraction was found to be rich in flavonoids by quantitative estimation and it was selected for this study.

Cell culture

Human keratinocytes (HaCaT) were grown in DMEM medium supplemented with 10% (v/v) Fetal Bovine Serum, 100 IU/ml penicillin and 100 mg/ml streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C. The cultured cells were passed every 72 h. The confluent culture was sub cultured using 0.25% of trypsin for 90s. Trypsin is inactivated using serum containing medium.

Cell viability assay[22, 23]

The effect of the ethanol extract and flavonoid rich fraction on HaCaT cell viability was assessed by (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. HaCaT cells were seeded at a density of 6 x 10^3 cells / well in a 96 well plate and incubated at 5% CO_2 at 37°C. After overnight incubation, the culture medium was replaced with fresh medium. Ethanol extract and flavonoid rich fraction at different concentrations (0, 1, 3, 10, 30 and 100 µg/ml) were added to each well respectively for 48 h. At the end of 48 h, 50µl MTT (5 mg/ml stock) was added to the cells and further incubated for 3 h at 37°C. The purple-coloured formazon crystals formed were dissolved in 100 µl of dimethyl sulfoxide by shaking at 400 rpm for 15 min at room temperature in a thermo shaker. The intensity of the colour developed was absorbed at 570 nm in a multimode micro plate reader. The percentage of cell viability was calculated when compared to a negative control. Percentage of viable cells at particular concentrations of extract and fraction was calculated by using the following formula:

Viability (%) = (Absorbance Test /Absorbance Control) × 100

Activity of ethanol extract and flavonoid rich fraction on morphology of HaCaT cells

The cell morphology in comparison to untreated cells was observed under phase contrast microscope (Leica, Germany) and photographs were taken at 40X magnification after 48h incubation.

RESULTS AND DISCUSSION

Cell viability assay was performed using HaCaT cells by treating them with different concentrations of Ethanol extract and flavonoid rich fraction. Flavonoid rich fraction of the *Dodonaea viscosa* induced a significant cell proliferation (104.84±1.5%) after 48 hours exposure at 10µg/ml and (112±3.1%) at 30µg/ml when compared to the control group. Flavonoids rich fraction showed significant proliferation up to 30µg/ml indicating its wound healing property. At higher concentrations (100µg/ml), the extract showed cytotoxicity. Ethanol extract showed mild wound healing activity when compared to flavonoids rich fraction. The significant activity in flavonoid rich fraction may be due to the presence of mixture of flavonoids such as quercetin, kaempferol and isorhamnetin which are already proved to have wound healing efficacy.

CONCLUSION

Dodonaea viscosa showed significant wound healing activity on HACAT cell line by cell proliferation assay. The present study provides the scientific evidence for the folklore claim of *Dodonaea viscosa* on wound healing. The cell proliferation assay can be used as a promising scientific approach and platform to evaluate plant extract known for their wound healing property.

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