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RESEARCH ARTICLE

Cytotoxicity and wound healing potential of jasmine essential oil

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SUMMARY

Jasminum sambac is an important fragrant and commercial flower crop of South India. Jasmine essential oil (JEO) is valued for its fragrance which is used mainly in the perfumery and cosmetic industry. JEO exhibits anti-inflammatory, anti-microbial activities and are used in the treatment of skin disorders. This present study was taken with the objective of understanding the cytotoxic and wound healing activity of JEO. JEO at different concentrations for cytotoxicity activity (0.5, 0.75, 1.0, 1.25 and 1.5 µg) and wound healing property (1.0, 1.25, 1.75 and 2.0 µg) were analyzed *in-vitro* for L929 skin cell lines. The results indicate that, JEO have moderate cytotoxic activity and lower wound healing property.

Key Words : Jasmine essential oil (JEO), Cytotoxicity, Wound healing

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Jasmine is an important traditional flower crop grown for its unique flower fragrance. *Jasminum sambac* is one of the important species of the genus *Jasminum* belonging to the family Oleaceae and is indigenous to India and Bhutan. It is commercially cultivated in South Indian regions especially Madurai is called as the Jasmine capital of India. Flower buds are used for making garlands, religious offerings and veni making (hair adornment). The flowers are also used for the production of attars and essential oils during glut. Jasmine essential oil (JEO) has a sweet and floral aroma. It is regarded unique, as it blends well with other floral extracts and which is highly valued throughout the world for its high grade perfumes. The flowers should preferably be picked early morning for extraction of essential oil. The plant exhibits anti-tumor (Radu and Kqueen, 2002), anti-microbial (Hussaini and Mahasneh,

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2009), anti-acne (Harisaranraj *et al*., 2010), anti-oxidant (Latif *et al*., 2010) and A.N.S stimulating effect (Hogratanaworakit, 2010). Roots are used to treat wounds and snake bites. The leaves and flowers have antipyretic and decongestant properties (Mourya *et al*., 2017). The flowers are used for treatment of diarrhoea, abdominal pain, conjuctivitis and dermatitis. The leaves and roots are used for treating diarrhoea, fever, pain and as an anaesthetic (Mourya *et al*., 2017 and Rahman *et al*., 2011). It is evident from various reports that leaves of *J. sambac* exhibits wound healing property while in flowers it has not been elucidated. *J. sambac* flowers are useful in diseases of the eye, ear and mouth, acting as tonic to the brain, purgative, allays fever, very good in toothache, suppurations, in diseases of blood, diseases of mouth, indolent ulcers, abdominal distention and diarrhea (Anonymous, 1949). Aroma-therapists find the Jasmine flower antidepressant and relaxing herb which is said to help with dry or sensitive skin and tiredness. In vapour therapy Jasmine oil can be useful for addiction, depression, nervousness, coughs, relaxation and tension. Decoction of dried flowers is used as eyewash during reddening and swelling pain in the eye, in cancers, conjunctivitis dermatitis, stomatopathy, opthalmopathy, prurities, cephalalgia, leprosy, hiccough, otopathy, vomiting, insanity, galactorrhoea (Sandeep and Paarakh, 2009 and Prajati and Kumar, 2003). Traditionally leaves are used in fever or cough, indolent ulcer, abdominal distension, diarrhoea, lowering the blood glucose level, regulating menstrual flow, to clean kidney waste, inflamed and blood shot eyes (Kiritikar and Basu, 2003, Nadkarni, 2007). The healing process can be physically monitored by assessing the rate of contraction of the wound (Sabharwal *et al*., 2012). JEO are a rich source of volatile organic compounds with amino acids which aid in anti-inflammatory which can be confirmed with the wound healing potential. This study was taken up with the objective of assessing the hydrodistilled JEO for its wound healing property and to investigate its cytotoxicity on skin cell line under *in-vitro* conditions.

MATERIAL AND METHODS

Extraction of essential oil :

Fully opened *J.sambac* flowers are harvested early in the morning and brought to the laboratoey under refrigerated condition. Essential oil was extracted through Clevenger apparatus (BRL – 3451029, India) with a sample solvent ratio of 1:2 for 3 h at a temperature of 50°C. The oil thus obtained was stored under refrigerated conditions at 4°C and used for analysis.

MTT cell viability assay:

The cells were grown in MEM medium supplemented with 10% FBS. The cells were trypsinized and counted on cell haemocytometer. Approximately 10,000 cells per well were seeded in a 96-well plate and incubate for 24 hours. The culture medium from the L929 cells was replaced with fresh medium. JEO at different concentrations *viz.*, (0.5, 0.75, 1.0, 1.25 and 1.50 µg) were added in triplicates on the cells. After incubation at 37±1°C for 18 h, MTT (1mg/ml) were added in all the wells and incubated for 4 h. After incubation, DMSO was added in the wells and read at 570 nm using photometer. Cytotoxicity and cell viability were analyzed as suggested by Mosmann, 1983 and calculated using the formula:

$$\text{Cytotoxicity} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \text{x100}$$

$$\text{Cell viability} = \frac{\text{Treated}}{\text{Control}} \text{x100}$$

In-vitro wound scratch assay:

As an *in vitro* model of wound healing assay, scratch test was performed to evaluate the ability of JEO to enhance the spreading and migration of fibroblast cells (Adetutu *et al*., 2011 and Fronza *et al*., 2009). L929 cells (5.0×103 cells/cm^2) were seeded in 6-well plates and when the confluent monolayer was obtained a linear scratch was generated using a sterile pipette tip. After that, cellular residues were washed out by PBS and 2 ml of fresh RPMI-1640 medium containing 4 and 16µg/ml (concentrations were selected from the results of the MTT assay) JEO was added to the wells. Plates were incubated at 37°C with 5 per cent CO_2 and photographs were taken at a 4x magnification on a single day at 0, 4, 18 and 24h. Using computing software Image J 147 the distance of each scratch closure was determined and percentage of healing was analyzed as suggested by Balekar *et al*. (2012).

Statistical analysis:

Experiments were conducted in triplicates and signicance at the level of 5% ($p < 0.05$) was considered.

RESULTS AND DISCUSSION

The results obtained from the present investigation

as well as relevant discussion have been summarized under following heads :

Effect of JEO on cytotoxic activities:

In the present study JEO was chosen to evaluate cytoxicity against skin cell line L929 at different concentrations (0.5, 0.75, 1.0, 1.25 and 1.5 µg). Cell cytoxicity decreases from 0.5 (52%) to 0.75 µg (46%) which gradually increased at 1.0 (54%), 1.25 µg (54%) and drastic increase of cell death was observed at 1.5 µg (64%). Cell viability was determined by MTT assay and the results indicate that cell viability was highest at 0.75 µg (54%). As shown in Fig. 1 JEO showed different cytotoxic activities toward the fibroblast skin cell line. A dose-dependent increase in the growth of cells was observed and the results suggest that JEO exhibited moderate cytoxicity toward the skin cell line when compared to standard. Novel natural products offer opportunities for innovation in drug discovery. Plant essential oil, its components and secondary metabolites have many applications in folk medicine. Essential oils are lipophilic compounds containing volatile aroma compounds.The constituents of the oils are mainly monoterpenes and sesquiterpenes. It has been reported that, JEO was evaluated *in vitro* for their anti-proliferative activity against Hep-2, MCF-7, and Vero cell lines. JEO

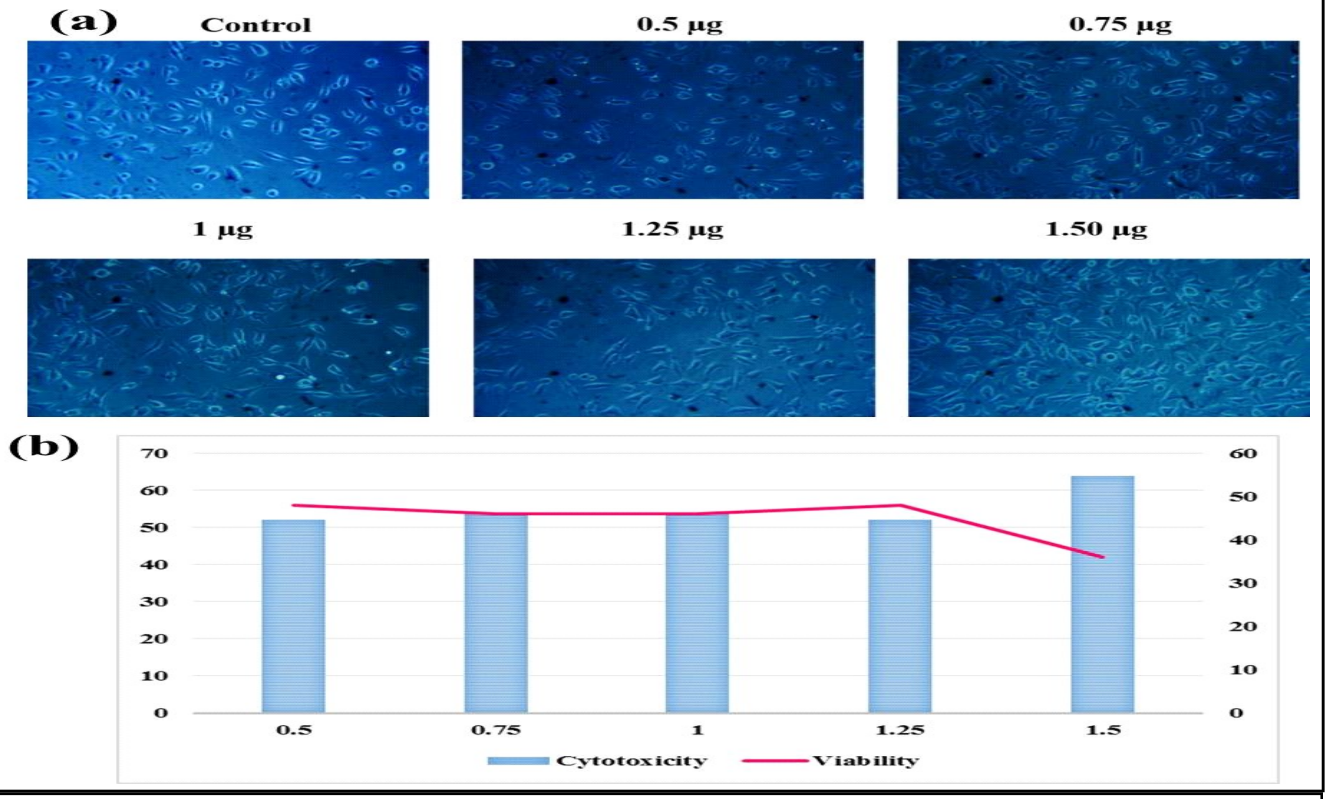

Fig. 1: (a) Effect of JEO on cytotoxicity using MTT assay and (b) graph representing cell cytotoxicity and cell viability on the L929 skin cell line

Table 1: Effect of JEO on the wound healing of L929 skin cell line

Sample name	Conc. (µg)	Wound area (µm)	Time interval (h) and percentage of healing (%)			
			0	4	18	24
Control		3752	0	64	95	>99
Jasmine essential oil	1	3152		0	14	31
	1.25	3541		0	11	24
	1.75	3569		0	7	11
	2	3482		0	0	0

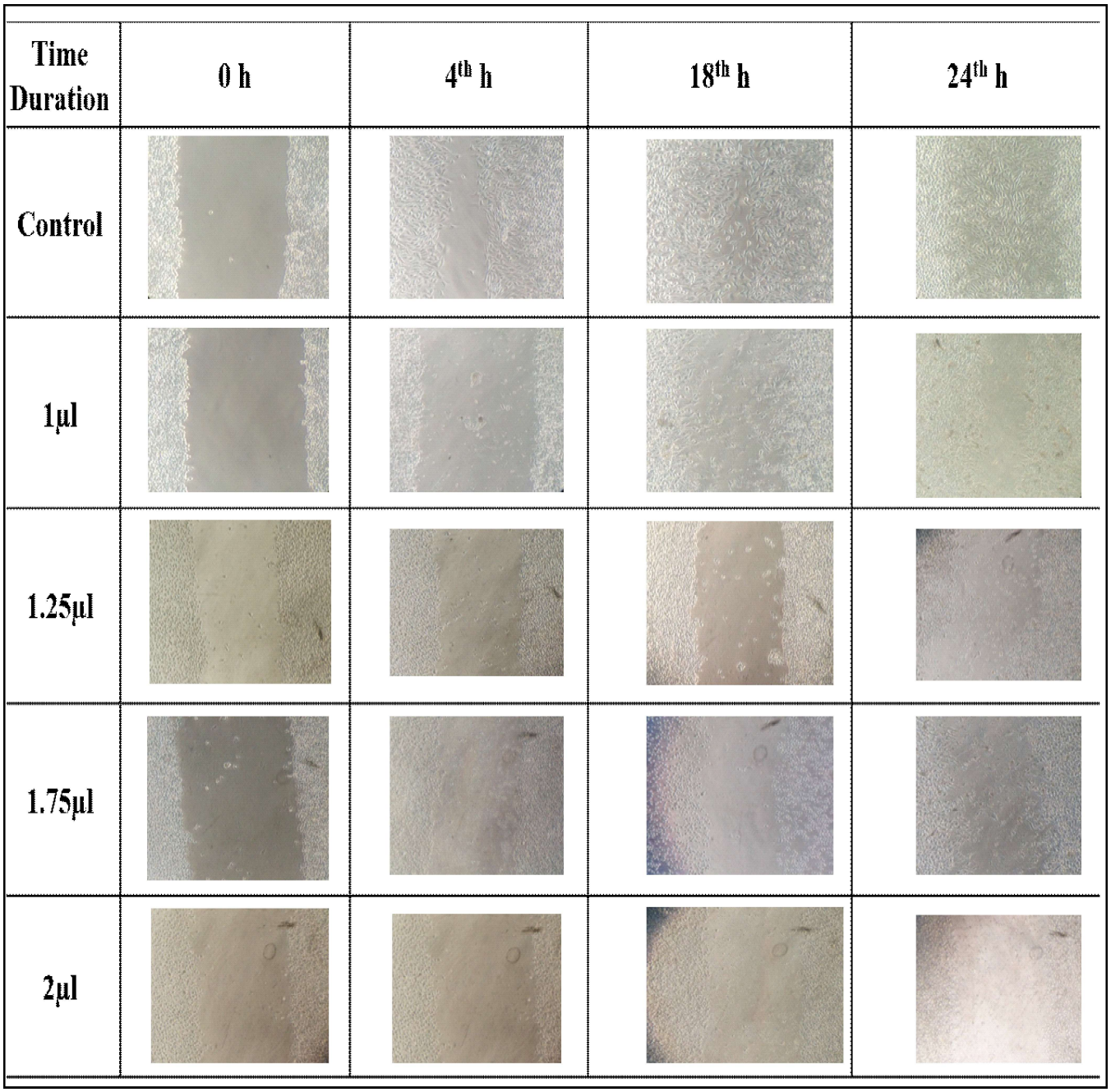

Fig. 2: Effect of JEO on mechanically induced wound on L929 skin cell line at different concentrations

exhibited significant anti-proliferative activity against one or more of the cell lines (Talib and Mahasneh, 2010). Essential oil behaves as lipophilic entity and passes through the cell wall and cytoplasmic membrane, causing a disruption of the cellular layer comprising of polysaccharides, fatty acids and phospholipids. Essential oil have also been reported to increase the membrane fluidity, this results in leakage of ions, (calcium ions) proteins, thereby leading to cell death by apoptosis and necrosis (Yoon *et al*., 2000 and Armstrong, 2006). JEO can also function as pro-oxidants by affecting the cellular redox status, leading to late apoptosis and necrosis.

Influence of JEO on the wound healing assay:

Wound healing process is an intricate procedure which concerns many events including angiogenesis, re-

epithelialization, granulation tissue formation and remodeling of extracellular matrix. Tests on the JEO revealed that this natural product has less topical healing effect. The results of the scratch assay model with JEO showed that JEO at 1 µg facilitated fibroblast migration in mechanically induced wounds, which was significantly lower than the control which indicates that JEO has less wound healing activity than the control. In the earlier days of healing process, fibroblasts proliferate and migrate to the wounded site. Hence, using fibroblast cells in the *in vitro* wound models, like scratch assay and evaluating is of significant value in studying the wound healing potential of natural products. On the other hand, assessment of proliferative activity or in contrast, toxicity of natural products against fibroblast cells, could be a valuable examination which somehow shows the potency and the safety of the tested product. This JEO has lower wound healing property because, cell proliferation decreases because of its heady and strong fragrance. It was also found that, at 2 µg concentration of JEO cell proliferation did not occur. *E. dysenterica* leaf extract promoted cell regeneration in HFF-1 fibroblasts after UVA exposure (Moreira *et al*.,2017). Extracts of *Calendula officinalis* and the triterpenoid faradiol myristate have also been shown to stimulate both the proliferation and migration of fibroblast (Fronza *et al*., 2009).

Conclusion:

The present study demonstrates moderate cytotoxic effect of JEO. JEOs regaining ability to induce proliferation, migration are lower and these findings corroborate its traditional use as a fragrant wound healing agent. A further phytochemical, *in vivo* study on the same has to be studied.

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