Original Article

ANNALS OF DENTISTRY
UNIVERSITY OF MALAYA

www.adum.edu.my

Ann Dent UM 2020, 27:50-54
DoI: 10.22452/adum.vol27no8

FLAXSEED (LINUM USITATISSIMUM) EXTRACT ACTIVITY ON HUMAN ORAL FIBROBLASTS (HOrF) CELL LINE

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KEYWORDS

L. usitatissimum, GC-MS, MTT, HOrF, wound healing assay

ABSTRACT

Natural products have demonstrated various activities beneficial to general health. Flaxseed (Linum usitatissimum) has been reported in many studies for its antimicrobial, antioxidant, and anti-inflammatory effects. Additionally, flaxseed extracts have skin wound healing activity and potential for treating oral ulcers. L. usitatissimum was extracted using 70% ethanol via soxhlet method and gas chromatography mass spectrum (GC-MS) was used to analyze the components of L. usitatissimum extract. The crude flaxseed oil were applied to human oral fibroblasts (HOrF), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to assess the cell viability after 24, 48 and 72 hours. Scratched HOrF cells were treated with crude flaxseed oil and healing was monitored per wound healing assay. GC-MS indicate that the major components present in L. usitatissimum oil extract are linolic, palmitic and oleic acid. L. usitatissimum crude oil extract showed high proliferation effect on HOrF cells at 24 and 48 hours, while the highest proliferation effect was recorded at 72 hours post-treatment. The wound healing assay results showed that healing activity of HOrF cells occurred as soon as 18 hours post-treatment when treated with L. usitatissimum crude oil extract. L. usitatissimum crude oil extract has proliferating and healing effects on HOrF cell line. Therefore, it can be considered as a potential promising oral wound healing agent.

INTRODUCTION

Flaxseeds (Linum usitatissimum) are the seeds derived of the flax herb characterized by its blue flowers. The seed's physical characteristics are flat, oval, 4–6 mm long, smooth surface, shiny brown color and about 4 to 6mm long [1].

Flaxseed plant has been around since 5000 BC; hence, it is considered as one of the oldest plants to survive modern day. Flaxseed is grown in the areas of West Asia and the Mediterranean. It is a plant rich in both oil and fiber; flaxseeds generally contain about 41% fat, 20% protein, 28% fiber, and 7.7% moisture [2]. In addition, it contains high amounts of omega-3 fatty acid, polyunsaturated linolenic acid, α-linolenic acid, phenolic compounds and lignan [3, 4 5]. Flaxseed has been reported to exert a wide variety of health benefits and forms an important part of the healthy functional food group; it is also considered safe for daily human consumption [6].

Flaxseed has many medicinal proprieties such as antioxidant, antiviral, antibacterial, antifungal, anti-inflammatory, antiatherosclerosis, anti-carcinogenic and chemo-preventive properties in animals and humans [7]. It prevents diabetic complications, has cardio-protective effects in rats [8], decreases menopausal symptoms and osteoporosis in women and many others [9].

Here we aimed to test the activity of flaxseed plant extracts on ATCC® human oral fibroblast (HOrF) cell fitness and viability and wound-healing potential.

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MATERIALS AND METHODS

Plant extraction

Whole flaxseeds were obtained from Faculty of Science, Philadelphia University, Amman Jordan. The seeds were crushed, grinded and made into a fine powder and then extracted using Soxhlet method with 70% ethanol solvent as a medium of extraction for 10-15 cycles. Then, the crude flaxseed oil was evaporated using rotary evaporator to remove the ethanol [10, 11]. The oil was preserved at 4 °C until ready to be used in analysis.

Gas chromatography-mass spectrum (GC-MS)

The flaxseed extract was analyzed using a ThermoQuest Trace GC 2000 connected to a Finnigan Trace MS quadrupole mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA). Peak retention times were recorded and compared against standards. The technique was used to demonstrate the major components of the crude flaxseed oil [12].

Cell culture and treatment

Human oral fibroblast (HOrF) cells obtained from ATCC® were cultured in 25cm^2 cell culture flasks in Dulbecco's Modified Eagle's medium supplemented with 10% Fetal Bovine Serum and 1% penicillin, and maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity [13]. After confluence, the cells were harvested, and cultured in 96-well plates. The wells were treated with crude flaxseed oil at 10, 11.1, 12.5, 14.2, 16.6, 20, 25, 33.3, 50 and 100 µg/ml concentrations, and left to incubate for 24, 48 and 72 hours before proceeding with MTT assay.

MTT assay

Cells treated with flaxseed oil after 24, 48 and 72 hours were tested using the colorimetric MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay to determine crude flaxseed oil toxicity on the HOrF cells. 20 µl of MTT solution was added to the wells and left to further incubate for 3 to 4 hours. Next, 150 µl of DMSO (dimethyl sulfoxide) was added to the wells and left to incubate for an hour. Absorbance was read using micro-plate reader (Tecan M200Pro, Austria) at 630 nm wavelength [14].

Wound healing assay

HOrF cells were grown in 6-well plates. Cells were treated with crude flaxseed oil at concentrations as mentioned above. A scratch (to mimic wound) was made in the cells in a straight line using a sterile pipette tip. The cells were then incubated in a 37°C 5% CO_2 incubator. The scratch area was monitored using a DinoXcope camera (Dino-Lite, Europe) attached to the inverted microscope. The cells were monitored after 18 hours of treatment to 3 - 4 days until either the scratch area was healed and covered with cells or the rest of the cells detached due to cell death [15].

Statistical analysis

Using Microsoft® Excel 11.0 (Office 2004) triplicate data for experiments was expressed as mean ± standard deviation (SD) [16].

RESULTS AND DISCUSSIONS

Flaxseed is an important oilseed crop that is important in the oleochemical, food and increasingly, functional food industries. Due to the unique composition, much research has been carried out on this plant seed. Some of the high value compounds present in flaxseed include the oil, proteins, lignans, and phenolics [17]. Different extraction methods have been explored to target different compounds present in flaxseed and to optimize yield.

Here, we report crude Soxhlet extraction of flaxseed oils using a 70% ethanol-water solvent, yielding 68% crude flaxseed oil. Binary solvent (alcohol-water) was reportedly more efficient for extraction of phenolics in flaxseed compared to a monosolvent extraction [18]. The difference in polarity between water and ethanol allows for extraction of different compounds in the flaxseed [19]. The use of ethanol-water mix allows for the extraction of important phenolics with high antioxidant activities [18] [19]. Previous studies have reported 70% and 80% ethanol to yield high amounts of flavonoids with superior antioxidant properties [20] [21]. Ethanol has become the preferred solvent for plant extractions with food application endpoints due to it being Generally Recognized as Safe for human consumption [22] [18]. Comparison between Supercritical fluid extraction solvent, Soxhlet and ultrasound-assisted extraction showed higher yields with Soxhlet extraction [23] [24] [25].

The crude flaxseed oil was analyzed using GC-MS to determine compounds present (Figure 1).

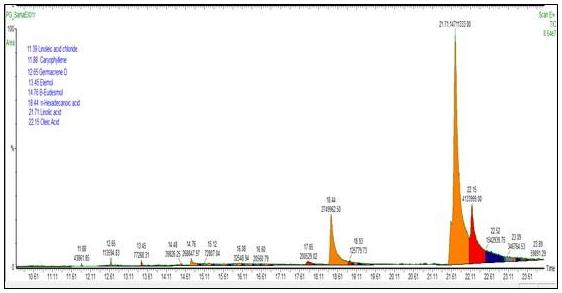

Figure 1: GC-MS peaks of the major components of crude flaxseed oil.

The highest peaks corresponded to larger amounts of the compounds. The compounds identified by GC-MS could be divided into two types: fatty acids (palmitic acid, linolic acid, linoleic acid and oleic acid) and sesquiterpenoids (caryophyllene, germacrene D, elemol and B-eudesmol) (Table 1). The highest peaks corresponded to linoleic acid, followed by oleic acid and palmitic acid. Virgin flaxseed oil is reportedly abundant in terpenes and sesquiterpenes, which have important biological activities, including antioxidant activity [26]. High levels of oleic acid followed by linoleic acid, in addition to palmitic acid were also reported in flaxseed extracts when analyzed using GC-MS [27].

Table 1: Retention times and compounds identified in GC-MS analysis of the crude flaxseed oil.

Retention Time	Compound
11.39	Linoleic Acid Chloride
11.88	Caryophyllene
12.65	Germacrene D
13.45	Elemol
14.76	B-Eudesmol
18.44	N-Hexadecanoic Acid
21.71	Linolic Acid
22.15	Oleic Acid

HOrF cells were treated with crude flaxseed oil at concentrations ranging from 10 µg/ml to 100 µg/ml, and assayed for viability using MTT assay [13]. The cells were observed at 24-, 48- and 72-hour post-treatment (Figure 2). We observed high proliferation in cells treated with crude flaxseed oil as soon as 24-hour post-treatment compared to cells with no treatment. Increased cell proliferation was observed with increased crude flaxseed oil concentration applied. Longer treatment times with crude flaxseed oil also resulted in higher proliferation rates; at 72-hour post-treatment, highest cell proliferation rates were recorded compared to cells with no treatment. These findings indicate that crude flaxseed oil is not only safe in HOrF cells but can also accelerate proliferation of the HOrF cells in a concentration- and time-dependent manner. The mean ± SD was calculated from the triplicate reading of each concentration in each time line.

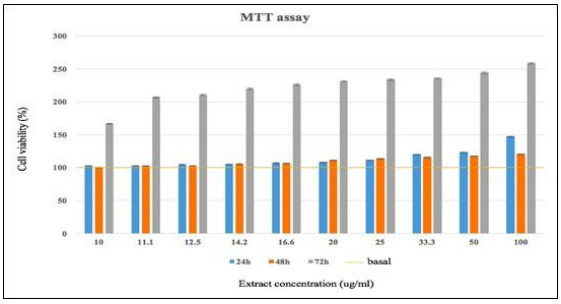

Figure 2: HOrF cell viability after 24, 48 and 72 h treatment with different concentrations of crude flaxseed oil with mean ± SD marked.

Wound healing assay (Figure 3) was performed by making a straight-line wound/ scratch using a pipette tip on confluent HOrF cells. The cells were then treated with crude flaxseed oil consisting of the fatty acids and sesquiterpenes.

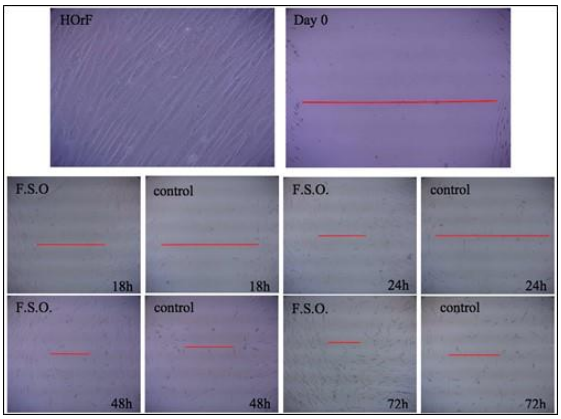

Figure 3: Wound healing assay on human oral fibroblast (HOrF) cells using flaxseed oil as treatment (F.S.O.) and no treatment for control.

The results were observed in a time line. The results showed that the cells re-grew as fast as 18-hour post-treatment with crude flaxseed oil compared to cells with no treatment. The treated cells continued to re-grow in the wound area until full coverage of the wound was achieved within 72-hour post-treatment in most areas of the wound. In contrast, HOrF cells with no treatment under the same conditions did not show any wound closure at the same time given. The findings suggest that crude flaxseed oil have high wound healing potential on human oral fibroblast cells, and therefore, it would be considered as natural oral wound healing agent for oral wounds. In another study, crude flaxseed oil

showed good healing action in burn wounds in rabbits with rapid healing times and better wound contraction [28] [29]. Terpenoids promote the wound healing process due to their astringent nature, while the fatty acids improve skin moisture to facilitate healing [28]. Additionally, oleic and linolic acid have been reported to promote the wound healing process and induce protective effects in cells [30].

CONCLUSION

Flaxseed (Linum usitatissimum) crude oil obtained from ethanol extraction contains a high amount of oleic, linoleic, and palmitic acids. Flaxseed crude oil has proliferative effects on human oral fibroblast cells, which are involved in wound healing. We find that wound healing time is improved with concentration-dependent application of the flaxseed oil compared to no treatment. Hence, crude flaxseed oil can be considered a promising natural healing agent with potential for healing wounds that result from dental procedures, such as tooth extractions.

ACKNOWLEDGEMENT

The project was sponsored by special grant project number: SP17-026-0288. All laboratory work was performed at International Islamic University Malaysia.

DECLARATION OF INTEREST

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article. The authors report no conflicts of interest.

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Editorial History

Date of Submission: 29 August 2020

Review and Revision: 01 September 2020 to 07 October 2020

Accepted: 04 November 2020

Published: 23 November 2020