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Review

Systematic review of flaxseed (*Linum usitatissimum* L.) extract and formulation in wound healing

[Revisión sistemática sobre extracto y formulación de linaza (*Linum usitatissimum* L.) en la cicatrización de heridas]

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Abstract

Context: Flaxseed constituents provide to antioxidant, anti-inflammatory, antimicrobial and wound healing benefits.

Aims: To systematically review the experimental evidence on the wound healing ability of flaxseed extracts and formulations.

Methods: Comprehensive searches in six databases (Scopus, Science Direct, Web of Science, PubMed, Google Scholar and Dimensions) were carried out from the beginning of databases until December 2020, according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. The terms used in searches were (*Linum usitatissimum L.*, flaxseed, linseed, flax) AND (extract) AND (wound heal, heal, heal type, wound) for collection of articles, with only articles in English and research articles were included. Transgenic term were excluded. AXIS tool was chosen to assess the quality and risk of bias. The data were then categorised in term of extracts, laboratory formulation, and wound healing.

Results: In total, 999 articles were collected and screened based on the pre-determined inclusion and exclusion criteria. Finally, 10 articles were included in the review. The majority of publications reported significant findings of flaxseed oil on wound healing regardless of extraction method and formulation. Healing parameters on excision, incision, and burn wound models were studied. Lack of laboratory formulation mentioned in the collected articles gave limitation impact on this study.

Conclusions: Flaxseed oil formulation appears to exert a positive effect on wound healing. Therefore, extensive studies needed to evaluate the transportation of flaxseed phytochemicals into skin dermis by advanced drug formulation.

Keywords: extraction; flaxseed; formulation; *Linum usitatissimum L.*; systematic review; wound healing.

Resumen

Contexto: Los componentes de la linaza proporcionan beneficios antioxidantes, antiinflamatorios, antimicrobianos y de cicatrización de heridas.

Objetivos: Revisar sistemáticamente las evidencias experimentales sobre la capacidad de cicatrización de heridas de los extractos y formulaciones de linaza.

Métodos: Se realizaron búsquedas integrales en seis bases de datos (Scopus, Science Direct, Web of Science, PubMed, Google Scholar y Dimensions) desde el inicio de las bases de datos hasta diciembre de 2020, según Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) protocolo. Los términos utilizados en las búsquedas fueron (*Linum usitatissimum* L., flaxseed, linseed, flax) AND (extracto) AND (herida curar, curar, curar tipo, herida) para la colección de artículos, con solo artículos en inglés y se incluyeron artículos de investigación. Se excluyeron los términos transgénicos. Se eligió la herramienta AXIS para evaluar la calidad y el riesgo de sesgo. Luego, los datos se clasificaron en términos de extractos, formulación de laboratorio y cicatrización de heridas.

Resultados: En total, se recopilaron y seleccionaron 999 artículos según los criterios de inclusión y exclusión predeterminados. Finalmente, se incluyeron 10 artículos en la revisión. La mayoría de las publicaciones informaron hallazgos significativos del aceite de linaza en la cicatrización de heridas, independientemente del método de extracción y la formulación. Se estudiaron los parámetros de curación en modelos de escisión, incisión y quemaduras. La falta de formulación de laboratorio mencionada en los artículos recopilados dio un impacto de limitación en este estudio.

Conclusiones: La formulación de aceite de linaza parece ejercer un efecto positivo en la cicatrización de heridas. Por lo tanto, se necesitaban estudios extensos para evaluar el transporte de fitoquímicos de la linaza a la dermis de la piel mediante una formulación avanzada de fármacos.

Palabras Clave: extracción; cicatrización de herida; formulación; *Linum usitatissimum*; revisión sistemática; semilla de lino.

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INTRODUCTION

Wound healing is a physiological cell proliferation process to restore the damaged tissue architecture and function after an injury. The damaged tissue will be replaced with new healthy living tissue, and the process begins within 24 h. Four main stages govern the wound healing process – haemostasis, inflammation, cell proliferation and contraction of the collagen lattice, including the formation of granulation tissue, re-epithelization and construction of new extracellular matrix and tissue remodelling within 2 to 4 weeks (Farahpour and Fathollahpour, 2015; Rafiee et al., 2017). Acute inflammation occurs within 24 h of tissue injury, and neutrophils are the predominant cell type, which is later replaced by macrophages. During this haemostasis phase, a clot will form to prevent bacterial infection and blood loss. After that, macrophages and neutrophils will remove the harmful agents and necrotic cells from the body. The formation of new blood vessels (neovascularization) will supply nutrients for the newly generated cells (Gonzalez et al., 2016).

A systematic review implemented by Olsson et al. (2019), observed that chronic wound imposed low health-related quality of life, together with cost burden for wound management. In fact, the complexity of wound healing cascade also limits the advance research of wound such as precise animal model, and comorbidities exist (Zhao et al., 2016). However, technologies developed to accelerate wound healing from systemic to local dressing-free therapies, to cell-free dressings. Subsequently, wound can be healed pharmacologically by natural compounds exhibits antibacterial, angiogenic, and regenerative effect despite of its drug carrier such as liposomes (Las Heras et al., 2020). Numerous studies on natural products has been performed for wound healing because of the bioactive phytochemical constituents such as alkaloid, essential oils, flavonoids, tannins, saponins, and phenolic compounds (Ibrahim et al., 2018). The treatment of wound with phytochemicals is considered to be choice of practice due to cost effective, wide distribution of plant across global region, and fewer or no known side effects of phytomedicines (Chingwaru et al., 2019).

Linum usitatissimum L., also known as flaxseed, is a herb in the *Linaceae* family. Its antioxidant and anti-inflammatory properties are believed to aid in wound healing (Draganescu et al., 2015; Rafiee et al., 2017). Many studies discuss biologically active compounds in flaxseed that contribute to the wound healing process such as omega 3 fatty acids (Jurić et al., 2020) and flaxseed carbohydrates (Trabelsi et al., 2020). In addition, the phenolic compound is also one of the abundant components found in flaxseed, such as caffeic acid, p-coumaric acid, and ferulic acid, together with the presence of secoisolariciresinol diglucoside (SDG) impedes to antioxidant properties for wound healing process (Wang et al., 2017). Reactive oxygen species (ROS) such as superoxide anions prevents or slow down the wound healing process by damaging the cellular structure. Antioxidant molecules present in flaxseed can protect the healthy cell by sequestering the ROS hence preventing oxidative stress (Draganescu et al., 2015; Rafiee et al., 2017). During adenosine triphosphate (ATP) production, the premature leak of electrons to oxygen was one of the factors for free radical production. As a result, superoxide anions were formed from the premature leak of electrons in the electron transport chain during aerobic respiration and slow down the healing process of a wound as they will damage the cell's DNA (Atkin et al., 2019).

This study fills in the gaps by systematically analysing previous relevant research in order to better understand the relationship between flaxseed extracts and their formulation on wound healing. The central research question guides the review: What are the flaxseed extracts and formulation wound healing abilities? This review describes the knowledge of flaxseed extract, typical drug formulation and its effect on wound healing.

MATERIAL AND METHODS

Review protocol

This systematic review was conducted following the PRISMA protocol (McInnes et al., 2018).

Formulation of research question and systematic searching strategies

The research question for this study was developed by using PICO (Mohamed Shaffril et al., 2021), a tool that helps the authors formulate an appropriate research question. Three main concepts in PICO are Population, Intervention, Control, and Outcomes, which in this study, the authors came out with the following aspects – flaxseed extract (Population), flaxseed extract drug formulation as a wound healing agent (Intervention), wound (Control) and wound

healing (Outcome), as described in Table 1. This PICO concept served as a guide for the authors to formulate the research question – what are the wound healing ability of flaxseed extracts and formulation?

Systematic searching strategies are briefly presented in Fig. 1.

Identification

The identification process begins with developing the main keywords based on the research question in order to retrieve all related publications from the selected databases. Synonyms and associated terms were used to formulate the keywords that later would be augmented into a full search string. Then, the search string was modified according to the format required for each particular database, as in Table 2. Six databases were included for data collection – Scopus, Science Direct, Web of Science, PubMed, and Google Scholar and Dimensions.

Science Direct and Scopus provide the advantage of covering the theme of the search terms precisely. Nevertheless, Scopus is more superior in terms of the number of citations. In addition, PubMed is more distinctive, especially in medical-related topics (Tober, 2011), despite of its Best Match algorithm that leads to significant improvement for relevant articles over times (Fiorini et al., 2018). Google Scholar was chosen as a supporting database because it provides comprehensive coverage in various fields and categories. However, its scholarly impact is slightly inferior due to the inclusion of lower quality citing documents despite higher citation counts than Scopus and Web of Science (Martín-Martín et al., 2018). Eventhough Dimensions is a new scholarly database exist, its improvement in dataset and user interface can lead to numerous data of articles that might be beneficial to be included in this study (Hook et al., 2018).

Screening

The screening process involved all articles retrieved from six databases, as mentioned during the identification stage previously. The initial step was conducted using the sorting or filter function that can be accessed on each database. This feature helped the authors to ease the initial screening process that involved hundreds of articles by automatically sorting the results based on criteria of interest set by the authors. The search was limited to publications only in the English language with no restriction for years of publication until December 2020. Furthermore, only research articles were incorporated in this study which, publications such as review articles, chapter in books, book series, books, news, magazines, patents and conference proceedings were excluded. Table 3 summarises the inclusion and exclusion criteria.

Eligibility

The third step in the systematic searching strategy is eligibility, which aims to ensure the selected remaining articles that have undergone the screening process are consistent with the inclusion criteria established by the authors. This process was manually done by reading the full-text articles. The articles that did not mention the main scope of the study, for instance, flaxseed, flaxseed extract and wound healing, were excluded. Furthermore, articles discussing the transgenic flax were also omitted because the term "transgenic" did not fulfil the requirement for this study.

Quality appraisal

Assessment of the quality of the selected articles was done independently by each author using the AXIS tool, which is a critical appraisal tool that evaluates the quality and risk of bias of a study. It was developed via the Delphi panel that consisted of 20 components (Downes et al., 2016). High, moderate and low-quality categories were decided to categorize the appraised articles, and only articles in high and medium categories were accepted. The papers that were ranked as low by all of the authors were rejected. If any of the authors ranked the article as low, all the authors would decide whether the article should be included or excluded until mutual agreement was achieved. During this stage, consensus of all authors was the principal strategy. Fig. 2 summarises the selected articles for appraisal.

Table 1. Criteria taken into account for the formulation of the research question using PICO.

Concept	Question
Participants/Population	Flaxseed (*Linum usitatassimum* L.)
Intervention/Indication	Formulations and extracts
Comparator/Control	Wounds
Outcome	Wound healing

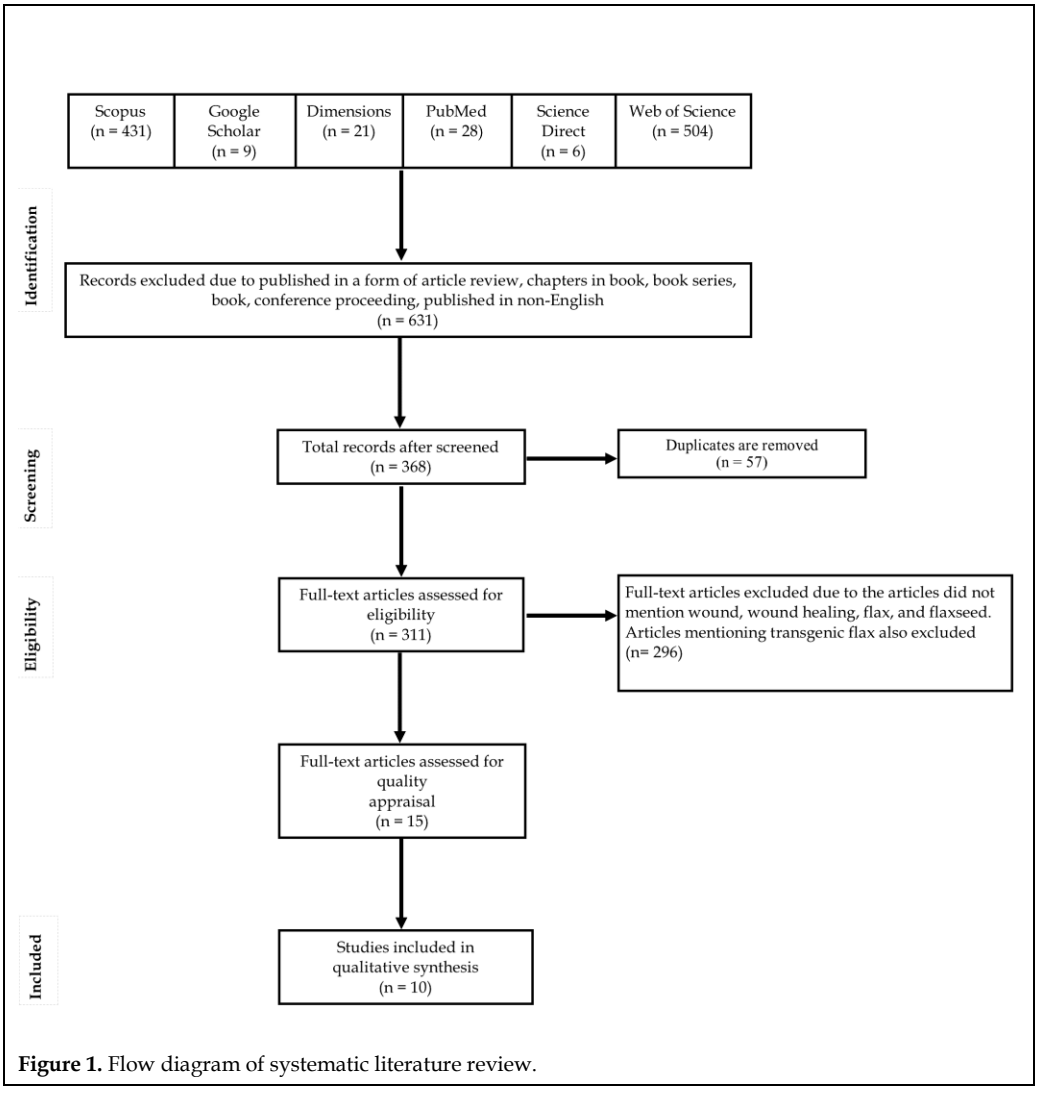

Figure 1. Flow diagram of systematic literature review.

Table 2. The search string for SLR.

Database	Search String
Scopus	TITLE-ABS-KEY (("Linum usitatissimum L." OR flaxseed* OR linseed* OR flax*) AND (extract*) AND ("wound heal*" OR "heal* type*" OR heal* OR wound*))
Science Direct	("Linum usitatissimum L." OR flaxseed OR linseed OR flax)(extract)(wound)
Web of Science	#1 TS="Linum usitatissimum L." OR TS=flaxseed* OR TS=linseed* OR TS=flax* #2 TS=extract* #3 TS="wound heal*" OR TS="heal type*" OR TS=heal* OR TS=wound* #4 #3 AND #2 AND #1
PubMed	("Linum usitatissimum" OR "flaxseed*" OR "linseed*" OR "flax*") AND (extract OR extracts OR extraction) AND ("wound heal" OR "wound healing" OR "heal type" OR heal OR wound)
Google Scholar	allintitle:("Linum usitatissimum L." OR flaxseed OR flax OR linseed)(extract OR extracts)(wound OR heal OR healthy OR wounds)
Dimensions	("Linum usitatissimum" OR "flaxseed*" OR "linseed*" OR "flax*") AND (extract OR extracts OR extraction) AND ("wound heal" OR "wound healing" OR "heal type" OR heal OR wound)

Table 3. The inclusion and exclusion criteria.

Criteria	Inclusion	Exclusion
Language	English	Non-English
Literature type	Research article	Review articles, chapter in books, book series, books, news, magazines, patents and conference proceeding
Scope	Articles mentioning flaxseed, flaxseed extract and discussing on wound healing study	Articles not mentioning flaxseed, flaxseed extract and wound healing. Articles discussing on transgenic flax.

Data abstraction and analysis

The authors thoroughly read articles selected for qualitative synthesis in order to identify the key data that were primarily discussed in the reviewed articles. The authors eventually came out with the following key data, which were structured in a standardized data collection form: (1) first author's last name; (2) year of publication; (3) flax type; (4) extraction method; (5) target compound; (6) formulation; (7) study model; (8) wound type; (9) dose; (10) variable; and (11) results. Then, the extraction and formulation are chosen as the main theme, following experimental study as the subthemes.

RESULTS AND DISCUSSION

Literature search results

The first step of systematic search yielded 999 articles obtained from Scopus (n = 431), Google Scholar (n = 9), Dimensions (n = 21), PubMed (n = 28), Science Direct (n = 6), and Web of Science (n = 504). After the exclusion of irrelevant articles (n = 631), 368 articles were screened. Fifteen full-text articles were appraised during the quality appraisal stage. Finally, 10 articles were determined to be eligible and completely relevant to include for the main purpose of this study. Their full texts were obtained for data processing. The data of

extraction method, formulation, and wound healing study are presented in Table 4.

The first article published regarding flaxseed on wound was in year 2011. There were 5 artilces in year 2015, and the article published mostly in every years until 2020. This indicate the interest on flaxseed research on wound healing, regardless on type of wound, extraction method, and parameter on wound healing.

Flaxseed extraction

In the successful articles collected, the experimental studies used flaxseed (n = 9) as their source of extracted compound, compared to flax fibre (n = 1). The extracted compounds were mostly obtained via the cold pressure method rather than cold-maceration and Soxhlet techniques. The target compound for wound healing generally consisted of phenolic compounds, including fatty acids, cannabidiol, carotenoid, lutein, and polysaccharides.

The seed, shives, and fibres give an average oil yield of 35-50% of the seed weight compared to fibres in about 15% yield. In fact, the variety of fibre, environment, and agronomic practices produced variables of oil yield (Zuk et al., 2015). High production yield is vital to optimise the processing activity from cultivation into the industrial market, besides its availability to enhance healthy dietary choices. Only one study used flax fibre, but it specifically mentioned cannabidiol as its target compound rather than phytosterols and flavonoids. Interestingly, the main objective elicited by this author is to look at the effect of cannabidiol on wound dressing due to its anti-inflammatory and antinociceptive effects (Styrcze-wska et al., 2015). However, the number of phytochemicals between flax fibre and seeds are comparative in terms of antioxidant activity (Wang et al., 2017) that contributes to wound healing. Still, the ease of harvesting and high production are the prominent factors.

In this study, we found that the cold pressure method is the most preferred method of extraction. It is consistent with the suggestion by Azwanida (2015) due to its applicable, convenient and less costly method for small and medium scale. However, we did not find the target compounds in this cold press to look at the variety of oil phytochemicals. Non-polar solvents such as hexane and petroleum ether were used to extract the flaxseed oil. This defatting method is one way to increase the percentage yield since the oil and protein are located at the endosperm of the seed (Zuk et al., 2015). Not all articles mentioned their intended compound for every extraction method, considering that the technique was established to produce phenols and fatty acids. Soxhlet by n-hexane has phenolics and fatty acids that act as antioxidants (Draganescu et al., 2015) and gives the yield oil of about 45.5% to 55.8% (Zou et al., 2017). In order to increase the yield percentage, Vieira et al. (2020) suggested utilising fresh solvent at a temperature close to the solvent boiling point. Only one article clearly mentioned that its study involved the function of alpha linolenic acid (ALA) as the substance intended in wound healing. ALA, which is also known as omega-3, is recognized to exhibit anti-inflammatory effects that are useful in healing cascade (Yadav et al., 2018). Despite non-polar solvent, one of the studies brought up the water-soluble polysaccharide as the target compound. The method of extraction used ethanol for pigment removal, then extracted at high temperature with deionized water. In summary, the extraction of flaxseed can be conducted using non-polar solvent and also polar solvent depending on the target compound intended for wound healing.

Flaxseed formulation

No similarity was discovered in flaxseed topical formulation, as the basics of fixed oil extracts, gel and cream were used as the medium of application. Two studies involved in the cream application, 1 study used ointment, 3 studies used gel, and 4 studies used their extracts without topical formulation. This study tries to look at drug formulation because of the importance of compounding in terms of correct pharmaceutical ingredient and excipient, considering formulation appearance, colour, pH, odour, chemical and physical stability to obtain optimum bioavailability and drug potency (Ghosh et al., 2019). Many topical applications are available worldwide, but simple formulations were used in these studies, which demonstrated positive therapeutic effects. However, our ability to compare the results across different formulations is limited due to a lack of information.

Author (Year)	Q1[a]	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Average Score[b,c]	Remark
Dogoury et al. (2014)	4	4	3	4	4	4	4	4	3	4	4	4	4	4	0	0	4	14.25	Moderate
Rafiee et al. (2017)	4	4	3	4	2	2	4	1	3	4	4	4	4	4	3	0	4	13.50	Moderate
Vogl et al. (2013)	4	4	0	4	4	4	0	2	0	0	4	4	1	4	0	0	0	8.75	Low
Styrczewska et al. (2015)	4	4	1	4	4	4	4	4	3	4	4	4	4	4	1	0	0	13.25	Moderate
Draganescu et al. (2015)	3	3	1	4	4	3	4	4	0	4	4	4	4	4	1	0	4	12.75	Moderate
Abruzzo et al. (2020)	4	4	0	4	4	4	4	4	1	4	4	4	4	0	1	0	0	11.50	Low
Al-Ahmad et al. (2020)	4	4	3	4	4	4	3	3	0	4	4	4	4	4	3	3	4	14.75	Moderate
Haseeb et al. (2017)	2	2	2	3	2	0	0	2	2	3	3	4	3	3	2	0	2	8.75	Low
Deng et al. (2020)	4	4	0	4	2	1	4	4	2	4	4	4	4	4	0	0	4	12.25	Low
Trabelsi et al. (2020)	4	4	2	4	3	0	4	4	3	4	4	4	4	4	0	0	4	13.00	Moderate
Paladini et al. (2015)	4	4	1	4	3	3	3	3	0	4	4	4	4	4	3	0	0	12.00	Low
Jabbar et al. (2019)	4	4	3	4	3	2	4	2	3	4	4	4	4	2	4	0	0	12.75	Moderate
Bardaa et al. (2016b)	4	4	3	4	4	4	4	4	3	4	4	4	4	4	0	0	4	14.50	Moderate
Bardaa et al. (2016a)	4	4	3	4	4	4	4	4	3	4	4	4	4	4	0	0	4	14.50	Moderate
Datta et al. (2011)	3	4	3	4	4	1	4	4	3	4	4	4	4	4	0	0	4	13.50	Moderate

Q1. Were the aims/objectives of the study clear?
Q2. Was the study design appropriate for the stated aim(s)?
Q3. Was the sample size justified?
Q4. Was the target/reference population clearly defined? (Is it clear who the research was about?)
Q5. Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?
Q6. Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?
Q7. Were the risk factor and outcome variables measured appropriate to the aims of the study?
Q8. Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?
Q9. Is it clear what was used to determined statistical significance and/or precision estimates? (e.g., p-values, confidence intervals)
Q10. Were the methods (including statistical methods) sufficiently described to enable them to be repeated?
Q11. Were the basic data adequately described?
Q12. Were the results internally consistent?
Q13 Were the results presented for all the analyses described in the methods?
Q14 Were the authors' discussions and conclusions justified by the results?
Q15 Were the limitations of the study discussed?
Q16 Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?
Q17. Was ethical approval or consent of participants attained?
[a]Questions 7, 13, and 14 in AXIS tool are not included in the appraisal because it do not fit the criteria for study
[b]Articles with average score below 12.5 are considered low, 12.5 until 15 are moderate, and above 15 are high
[c]The scores average scores are calculated of means of 4 authors in quality appraisal

Figure 2. Selected articles for quality appraisal.

Table 4. Extraction of flaxseed oil, formulation and wound healing models.

Article No.	Authors	Year	Flax type	Extraction method	Target compound	Formulation	Study model	Wound type	Dose	Variable	Results
1	Datta et al.	2011	Seed	Arpitha Aromatics, Bangalore, India. Identified and confirmed by Pharmacognosy Department of Research and Development Center of Himalaya Drug Company, Bangalore, India.	Alpha linolenic acid	*Shorea robusta* resin extract in flaxseed oil (1:4) emulsion cream	Wistar rats	Excision and incision	Once daily	Wound contraction percentage, hydroxyproline content, breaking strength	85% wound contraction on day 9 post excision, 443 gms breaking strength, and 2.8 mg/100 mg tissue hydroxyproline on day 8 post incision.
2	Dogoury et al.	2014	Seed	Cold pressure	-	Flaxseed oil 2% in eucerin-vaseline ointment	New Zealand rabbits	Incision	Once daily	Wound contraction percentage, cell infiltration, angiogenesis, fibroblast distribution, collagen deposition	Up-regulated polymorphonuclear (PMN) on day 3, higher fibroblast distribution after 7 days, 81% wound healing on day 12.
3	Styrczewska et al.	2015	Fiber	Dew retting process.	Phytosterols, fatty acid, cannabidiol, carotenoid lutein	Fixed hydrophobic extract	Normal human dermal fibroblasat (NHDF), Normal human epidermal keratinocytes (NHEK)	*In vitro* wound scratch assay	-		Flax fiber extract can be part of a formulation to treat long-standing nonhealing wounds, anti-inflammatory and collagen production promotion properties of the extract are mainly due to the CBD content, acting synergy with beta sitosterol. Effect on matrix remodeling activity is mostly dependent on the phytosterol content.
4	Draganescu et al.	2015	Seed	Soxhlet extraction in hexane	Phenolics; SDG, SECO, LARI, MATA, PINO, t-ferulic acid, GAE, p-coumaric acid, fatty acid; oleic acid, palmitic acid, linoleic acid, linolenic acid	Cream of 4 g lyophilised flaxseed in 61 g flaxseed oil and 18 g beeswax	Wistar rat	Incision and excision	Once daily	Wound contraction percentage.	100% healing at day 20, including complete epithelisation with mature fibrous tissue and disappearance of collagen strands.
5	Bardaa et al.	2016a	Seed	Cold pressure	-	Fixed oil	Wistar rats	Laser burn	Once daily (0.52 $\mu L/mm^2$)	Wound contraction rate, collagen synthesis	0.15 ± 0.15 cm^2 of wound remain on day 7, Collagen density in flaxseed oil-treated group (62.60 ± 13.72) was the lowest among all groups.

Table 4. Extraction of flaxseed oil, formulation and wound healing models (continued...)

Article No.	Authors	Year	Flax type	Extraction method	Target compound	Formulation	Study model	Wound type	Dose	Variable	Results
6	Bardaa et al.	2016b	Seed	Cold pressure	-	Fixed oil	Wistar rats	Deep second-degree burn	Once daily every 2 day (0.52 µL/mm^2)	Wound area, wound contraction percentage, hematoxylin-eosin staning	Complete wound healing on day 33 (0.05 ± 0.012 cm^2), with 98.68% of wound contraction, 41.9 ± 5.54 collagen density. Linseed oil showed thick and well-structured epidermis covering entire area of burn wound.
7	Rafiee et al.	2017	Seed	Cold-macerated in petroleum ether	-	5% and 10% flaxseed hydroalcoholic in 2% carboxymethylcellulose (CMC)	Wistar rats	Excision	Once daily	Wound closure rate (100%), fibroblast distribution, collagen deposition, volume density, length density, and vessel diameter	9.43% per day wound closure for 5% flaxseed extract gel, 9.81% per day wound closure for 10% flaxseed extract gel, higher fibroblast distribution than control and base group, significantly higher length densities of vessel, and volume densities of collagen bundles.
8	Trabelsi et al.	2020	Seed	Pre-extracted with 95% ethanol at room temperature, then extracted with deionized water at 90°C, and precipitated with 95% ethanol producing water-soluble polysaccharide (LWSP) powder.	Heteropolysaccharide; glucose, mannose, xylose, arabinose	Gel of 15 mg/mL LWSP in water dissolved in glycerol 30%	Wistar rats	Laser burn	Once daily	Wound contraction rate percentage, collagen synthesis	LWSP accelerate burn wound starting on 3rd day. 98.16% burn wound healing on 8th day, high hydroxyproline (842.82 ± 5.44 mg/mg tissue).
9	Jabbar et al.	2019	Seed	Soxhlet by hexane, Dichloromethane (DCM) & methane.	-	100, 50, 25, 12.5 µg/mL in dimethylsulphoxide(DMSO)	New Zealand rabbits	Incision	Twice daily	Skin elasticity	Skin elasticity improved from 3.46 on day 1 to 2.46 on day 14.
10	Al-Ahmad et al.	2020	Seed	-	-	Fixed oil	New Zealand rabbits (diabetic induced)	Incision	- -	Wound contraction rate, reepithelization, cell infiltration, vascularization, vessel formation	Flaxseed treated group showed higher number of fibroblast cells proliferation for angiogenesis and granulation tissue deposition than control group in non diabetic-induced rabbit at day 14 interval. Faster healing with significant decrease in the severity of inflammation of non-diabetic animals at 7th day interval and 14th day interval for diabetic animals. In flaxseed exposed group both diabetic and nondiabetic animals, the epithelization and surface closure rate were more observable than that of control by days 4 and 7, respectively.

Only one study transports the phenolics compound across the dermis to further enhance the wound healing process (Draganescu et al., 2015). Flaxseed oil can also be used as a base in the cream formulation as experimented by Datta et al. (2011), where *Shorea robusta* resin and flaxseed oil (1:4 proportion) were formulated as the base. It is a promising formulation since the utilization of natural sources as a base can replace othercommercialized excipients, in addition to its anti-inflammatory and antioxidant properties. However, further study is needed to evaluate the formulation.

Experimental studies

In one *in vitro* study, normal human dermal fibroblasts (NHDF) and normal human epidermal keratinocytes (NHEK) were used to produce a hydrophobic extract containing phytosterols, fatty acids, cannabidiol, carotenoid, and lutein. *In vivo* experiment consisted of incision wound (n = 3), excision wound (n = 1), incision and excision wound (n = 2), laser burn wound (n = 2), and deep second-degree burn wound (n = 1) that were tested on New Zealand rabbits (n = 3) and Wistar rats (n = 6). A study on the animal model suggested that once-daily application was sufficient to improve wound healing with a shorter period, beginning at day 5 (Trabelsi et al., 2020). The longer period observed at day 33 for complete healing for once daily application alternate day (Bardaa et al., 2016b).

The excision wound model was the preferable method since it can evaluate wound healing potential, including epithelisation, area of wound contraction, wound index, and collagen deposition estimation comprehensively. All 9 studies assessed the effect of flaxseed extracts on the wound, either by direct observation or manual measurement, digital photography or software analysis. Parameters of wound healing were expressed either on wound healing percentage (100-(wound area on day X/wound area on day 0 × 100)), or percentage of wound healing rate (((area at visit 1 – area at each visit)/area at visit 1) × 100). The incision wound model was used for the assessment of success or healing potential (Shrivastav et al., 2018). Skin elasticity and breaking strength were experimented with in incision wounds throughout this study. Skin elasticity showed improvement from day 1 to day 14 (Jabbar et al., 2019), but the breaking strength was comparable with the control group (Datta et al., 2011). Burn wound model was also used to investigate wound contraction and collagen synthesis. Using H&E staining, flaxseed oil depicted a thick and well-structured epidermis covering the entire area of the burn wound on day 33 (Bardaa et al., 2016b).

On the whole, flaxseed oil is proven to offer healing effects by up-regulating cell infiltration, develops angiogenesis, increases fibroblast deposition and improves skin elasticity towards rapid wound contraction.

Limitations

There are several limitations that appear during this study, which may result in the less accurate analysis. First, the language selected in this study may limit the collection of the data. Second, only 10 collected articles analysed after following thorough PRISMA guideline, which may impede less analysis on the systematic review terms (extraction, formulation, and wound). Third, the transgenic flax, which is excluded in this study that may come into high impact data contributions. However, this is the first systematic review arises concerning the experimental analysis of flaxseed extracts, drug formulation, and its effect on wound healing.

Future perspectives

This study recommends the future analysis of flaxseed extracts on wound healing, especially involving the sophisticated drug formulation, and its effectiveness through wound healing mechanisms. It would also suggested to further investigate the differences of flaxseed types including transgenic flaxseed since the differences in traits may lead to improvement of biological compounds.

CONCLUSIONS

Experimental evaluation implemented in this study did demonstrate the positive effect of flaxseed oil in improving the wound healing process. However, the limitation regarding the small number of studies available was taken into account. More studies in small or especially large animal models with respect to suitable drug formulations are needed for solid conclusion. Though, this systematic review suggests for the participation of advancement in topical drug formulation to further enhance the healing of the wound.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Mat Sharil AT	Basma Ezzat M	Widya L	Amri Nurhakim MH	Nor Hikmah AR	Nabilah Zafira Z	Haris MS
Concepts or ideas	x			x	x	x	x
Design	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x				x
Literature search	x			x	x	x	
Data acquisition	x			x	x	x	
Data analysis	x			x	x	x	
Statistical analysis	x			x	x	x	
Manuscript preparation	x			x	x	x	x
Manuscript editing	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x

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