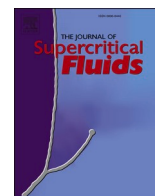




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## Advancements in conventional and supercritical CO<sub>2</sub> extraction of *Moringa oleifera* bioactives for cosmetic applications: A review

Júlia C. Kessler<sup>a,b,c,\*</sup>, Isabel M. Martins<sup>a,b</sup>, Yaidelin A. Manrique<sup>a,b</sup>, Alírio E. Rodrigues<sup>a,b</sup>, Maria Filomena Barreiro<sup>c</sup>, Madalena M. Dias<sup>a,b</sup>

<sup>a</sup> LSRE-LCM - Laboratory of Separation and Reaction Engineering–Laboratory of Catalysis and Materials, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal

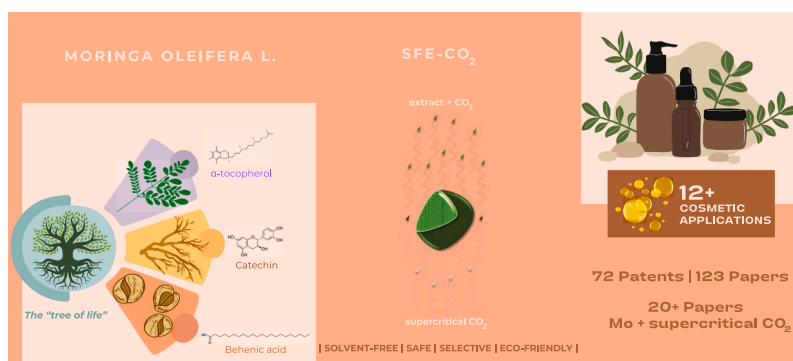
<sup>b</sup> ALiCE - Associate Laboratory in Chemical Engineering, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal

<sup>c</sup> Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal

## HIGHLIGHTS

- Mo properties benefit cosmetics, being explored in 72 patents and 123 publications.
- Anti-aging and photoprotective effects of Mo compounds stabilize skincare.
- Mo extracts promote wound healing.
- SFE-CO<sub>2</sub> optimizes Mo selectivity extract for high-quality cosmetic use.
- Pressure is key in extracting non-polar and low-polar compounds from Mo.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

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Supercritical CO<sub>2</sub> extraction, efficiency, stability, optimisation  
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 cosmetic ingredients

## ABSTRACT

*Moringa oleifera* L. extracts (Mo) have attracted attention as a sustainable and effective alternative to synthetic ingredients for cosmetic formulations. The unique and diverse phytochemical profile of the Mo tree enhances the quality and appeal of commercial products, as evidenced by numerous studies and patents. Supercritical carbon dioxide extraction (SFE-CO<sub>2</sub>) is particularly advantageous for this purpose, offering enhanced thermostability and selectivity of extracted compounds compared to conventional methods. This review examines the safety and efficacy of Mo seed, leaf, and root extracts as cosmetic ingredients, focusing on their bioavailability and performance by considering the thermodynamics and operational benefits of SFE-CO<sub>2</sub>. The collected data highlights the method's efficiency in terms of the total extraction yield and the recovery of target compounds from Mo, providing insights from optimisation studies and linking the solvation power of supercritical CO<sub>2</sub> with the significant non-polar and low-polar compounds present in Mo extracts.

\* Corresponding author at: LSRE-LCM - Laboratory of Separation and Reaction Engineering–Laboratory of Catalysis and Materials, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Porto 4200-465, Portugal.

E-mail address: [juliakessler@fe.up.pt](mailto:juliakessler@fe.up.pt) (J.C. Kessler).

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## 1. Introduction

New cosmetic formulations are being developed to meet market demands, based on popular acknowledgement of empirical results, supported by scientific evidence, to improve the skin appearance, such as its luminosity and viscoelasticity, as well as to reduce the epidermis oxidative stress, mainly noticed as anti-ageing and photoprotective effects [1]. Ayurveda has emerged from India as a millenary alternative medicinal system that has disseminated the benefits found in the *Moringa oleifera* L. (Mo) tree (Fig. 1a) [2]. Its rich bioactive composition has been confirmed by the antioxidant, anti-inflammatory, antibacterial, and antifungal activity [3], mainly related to phenolic acid, flavonoid, organic acid, terpene, alkaloid, tannin, fatty acid, saponin, and sterol compounds [4].

These bioactive properties introduce Mo as a potential ingredient for cosmetic formulations [5], varying its functionality according to the plant part and compounds' availability. For example, seed extracts (Fig. 1b) have been used for photoprotective [6], hydrating, enlightening, and anti-ageing purposes [5], while leaf (Fig. 1c) extracts have been used for epidermal and dermal inflammation [7], because of their high potential against immune disorders [8]. Antimicrobial and anti-inflammatory activity has been found in Mo root extracts [9,10] (Fig. 1d), but there is no evidence of its use in cosmetic formulations. Table 1 presents some of the molecules responsible for the bioactive properties of Mo plant parts. [11]

Regarding its bioavailability, two main influences can be listed:

cultivation conditions [12] and extraction methods [13,14]. The first one is related to geography, weather, and genetic variations that may affect the synthesis and extractable amount of bioactives [15,16]. These variabilities can influence the well-known benefits of Mo-based products [12], explained by the plant biological system. Under environmental stress (cool climates), the plant increases its demand for the antioxidant properties of phenolics as self-protection and, consequently, increases its synthesis of unsaturated fatty acids [16]. This mechanism occurs between seasons, and it is evidenced in the phytochemical variation presented in the literature [14,17–19]. However, the Mo tree has excellent adaptability to different soils and climates, being easily propagated and maintained [20].

To assess its potential, the chosen extraction methodology highly influences the ability to improve or degrade the compounds [21]. Therefore, this review focuses on the extraction of Mo compounds to use as an active ingredient in cosmetic formulations. The next section is dedicated to accessing the phytochemical composition of Mo seed, leaf, and root and its benefits to dermatological products. The third section reviews scientific publications, patents, and commercial cosmetics containing Mo extracts. The fourth section accomplishes a summary of the recent updates of conventional and alternative extraction methodologies, mainly regarding the emerging supercritical carbon dioxide extraction (SFE-CO<sub>2</sub>). Its operational parameters, such as pressure, temperature, use of co-solvent, particle size, and extraction time, have been studied to clarify the respective effects on extraction yield and Mo extracts' composition. Finally, the fifth section shows optimisation

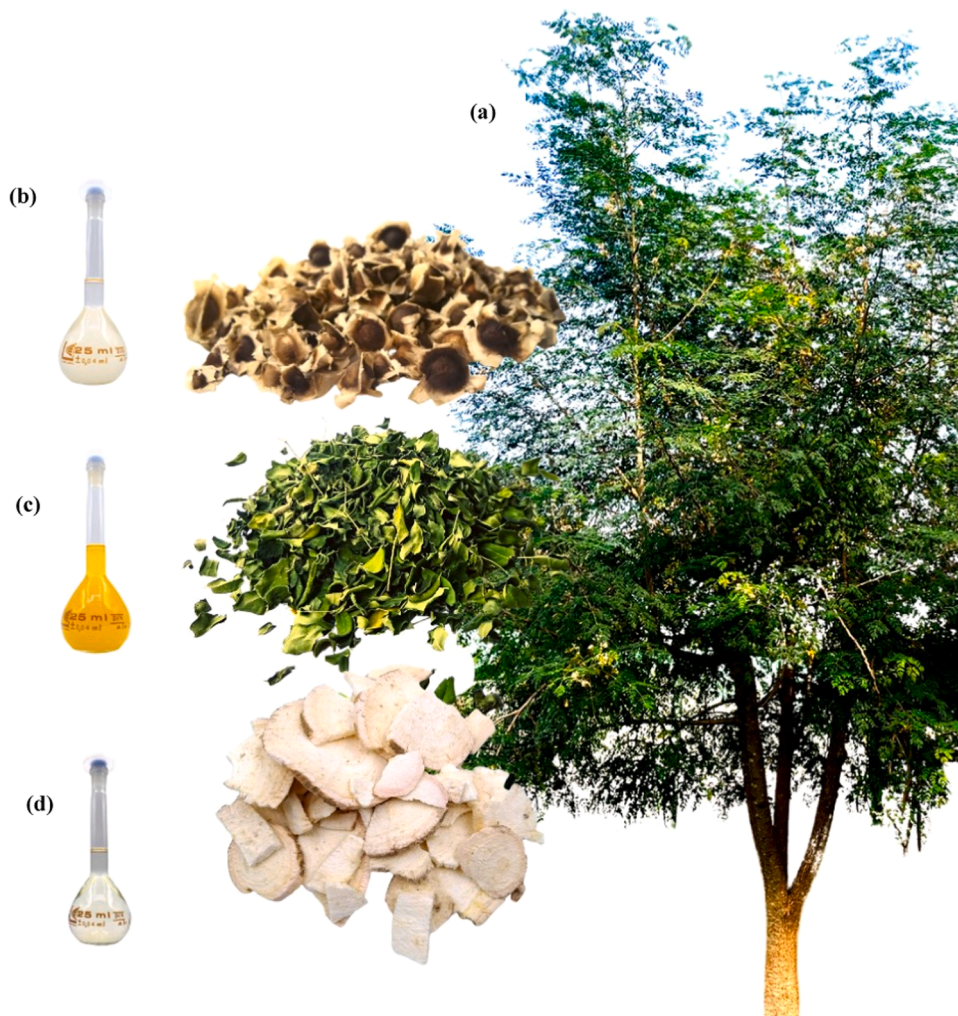
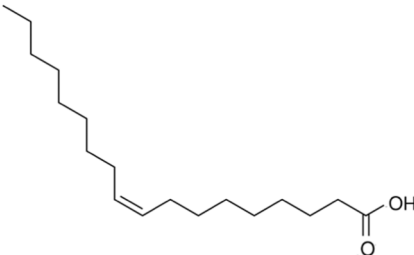
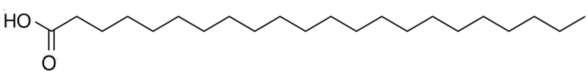
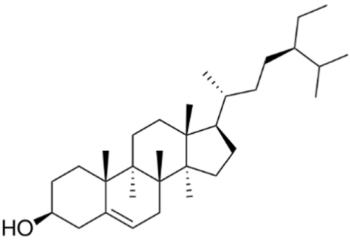
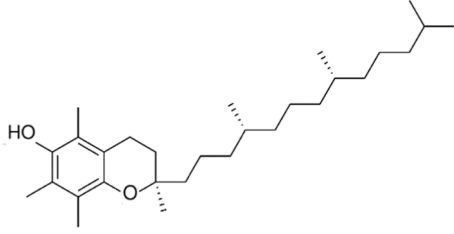
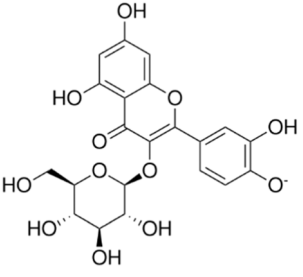
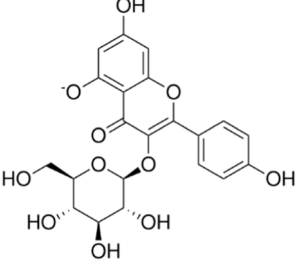
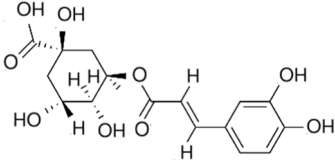


Fig. 1. (a) Mo tree and respective dry extractable parts: (b) seed, (c) leaf, and (d) root, and their respective extracts in solution obtained by SFE-CO<sub>2</sub>.

**Table 1**  
Molecular structure and polar surface area of phytochemical extracted from Mo seed, leaf, and root.

Plant part	Name	Class	Chemical structure	Polar surface area (Å <sup>2</sup> ) [11]
Seed	Oleic acid	Fatty acid		37.3 (PubChem CID 445639)
	Behenic acid			
	β-sitosterol	Sterol		20.2 (PubChem CID 1107)
Seed and leaf	α-Tocopherol	Tocopherol		29.5 (PubChem CID 2116)
Leaf	Quercetin-3-O-D-glucoside	Flavonoid		127.0 (PubChem CID 44259136)
	Kaempferol 3-O-glucoside			186.0 (PubChem CID 5282102)
	3-Caffeoylquinic acid	Phenolic acid		165.0 (PubChem CID 1794427)

(continued on next page)

studies using statistical strategies to ensure the obtainment of high-quality Mo extracts.

## 2. The tree of life: Mo biocompounds and its strategic use

Mo is a perennial angiosperm tree, originally from the sub-Himalayan region, disseminated worldwide, especially in tropical climates [3]. Its leaves, fruits, flowers, seeds, and roots are rich sources of flavonoids, phenolic acids, saponins, fatty acids, sterols, alkaloids, and macro and micronutrients [22]. Known as the “Tree of Life”, it has long been used in alternative, i.e. Ayurvedic medicine [23]. However, Mo extracts have received attention in the scientific field mostly in the last two decades. Its nutritional, bioactive, and healing potential have been the subject of several reviews [2,3,22,24–29]. Cosmetics products [5, 30–32], water treatment [33,34], and biodiesel production [35] are some examples of the wide list of Mo applications.

An extensive summary of the main phytochemicals obtained from the Mo tree and its specific biological activity was previously described [36]. The next sections highlight the main compounds and properties obtained from seed, leaf, and root extracts and their respective relevance in cosmetic formulations as active ingredients.

### 2.1. Mo seed: much more than a “ben oilseed”

Old civilisations have used Mo seed extract as an ingredient for perfume and skin lotion formulations [37]. Nowadays, cosmetic products are being developed due to the Mo seed’s attractive and functional properties, namely its emollient, nourishing, moisturising, and antioxidant activities [27]. Compounds such as oleic (C<sub>18:1</sub>) and behenic (C<sub>22:0</sub>) acids have shown a high lubrication ability, with relative concentrations above 80 % and 33 %, respectively [13,27]. Palmitic (C<sub>16:0</sub>), stearic (C<sub>18:0</sub>), and arachidic (C<sub>20:0</sub>) acids are also found in Mo seed extract composition [13]. The extract has a pleasant and peanut-like aroma, light yellow colour, and high thermal stability due to the low content of polyunsaturated fatty acids [5,38,39].

Mo seed extracts offer a vast diversity of bioactive compounds. In synergy with fatty acids, the sterol molecules are responsible for the anti-inflammatory activity, reflected in the skin’s anti-ageing effect [40]. The chemical group is represented, for example, by campesterol, campestanol, ergostadienol, stigmasteril,  $\beta$ -sitosterol,  $\delta$ -avenasterol, clerosterol, 24-methylenecholesterol,  $\delta$ -campestanol, stigmasterol, and 28-isoavenasterol molecules [41,42]. In addition, seeds are a natural reservoir of saponin compounds, identified as a precursor to the synthesis of steroid hormones [4].

The well-known antioxidant effect of Mo seed extracts results from the presence of  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -tocopherol [43], as well as the luteolin, quercetin, multiflorin B, quercetin-3-O-D-glucoside, and kaempferol-3-O-glucoside flavonoids [44,45]. The rich phytochemical profile includes tannin, phytate, alkaloid, and terpenoid molecules. Its nutritional composition comprises minerals (e.g. Ca, P, Mg, K, and Fe), vitamins (e.g. A, C, and E), and essential amino acids (e.g. isoleucine, leucine, phenylalanine, threonine, valine, alanine, and lysine) [46–48]. Other compounds identified in Mo seed extracts are

4-hydroxyphenylacetone nitrile, 4-hydroxyphenyl-acetamide, 4-(L-rhamnosyloxy) phenyl acetonitrile, and 1,4-( $\alpha$ -L-rhamnosyloxy) benzyl isothiocyanate. Recently, the anti-inflammatory evidence of four isothiocyanates found in Mo seeds has been categorised according to their inhibition mechanism [49].

Several studies performed with Mo seed have recognised its extract as an active ingredient for topical products. The literature emphasises the protective effect against skin oxidative stress promoted by UVB radiation due to the high content of phenolic compounds [6]. Furthermore, Mo seed extract formulations have increased skin hydration and reduced skin erythema, and these results were explained by the extract’s high antioxidant activity and by the high physical stability of the creams [5]. The skin-protecting pollution effect, usually introduced as an ingredient in skin and hair care products, has also been identified due to a unique peptide fraction [20]. In addition, Mo extracts have been applied to improve the skin’s pigmentation and enhance the skin’s natural glow [5,50].

Recently, encapsulated bioactives extracted from Mo seed were introduced into face masks as a response to their generalised use during the COVID-19 pandemic, having shown potential anti-allergenic, anti-inflammatory properties [51].

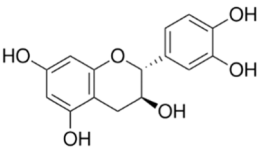
### 2.2. Mo leaf and the anti-inflammatory potential of its flavonoid molecules

The chemical composition of Mo leaf extracts explains its recognised bioactivity. The extract is a complex matrix rich in organic acids, phenolic acids, flavonoids, carotenoids, thioglycosides, and lignans [14, 36]. This herbaceous plant has been widely used due to its antioxidant and anti-inflammatory activities, which mainly result from phenolic compounds, with emphasis on the chemical group of flavonoids. Flavonoids have free (aglycones) and conjugated structures esterified with one or two sugar molecules (glycosides). They can be released through hydrolysis by breaking glycosidic bonds and then easily detected and quantified [52]. Quercetin and kaempferol stand out among more than twenty flavonoid molecules identified in Mo leaf samples from Madagascar [14]. Apigenin, luteolin, and myricetin were also extensively reported in Mo leaf extracts [29,53].

Due to the high number of phytochemicals, Mo leaf has shown potential in the therapeutic field. Regarding its anti-inflammatory activity, Mo leaf extract performed well against atopic dermatitis, an inflammatory skin disease [7]. Both levels of pro-inflammatory cytokine-related mRNAs and mitogen-activated protein kinases *in vitro* were reduced, as well as improved skin thickness. Moreover, isolated fractions of polyphenols and isothiocyanates have increased the anti-inflammatory response for lipopolysaccharide (LPS)-induced inflammation [54].

Bioactives extracted from Mo leaves were correlated to the mechanism of action of elastase, collagenase, and hyaluronidase enzymes [55]. These enzymes have been reported as responsible for skin ageing effects. Suitable interaction was evidenced between epigallocatechin gallate and both elastase and collagenase. In turn, 3-caffeoylquinic acid showed high activity against hyaluronidase. *N*-fructosyl, vicenin-2, and kaempferol-3-O-glucoside compounds have also indicated activity as

Table 1 (continued)

Plant part	Name	Class	Chemical structure	Polar surface area (Å <sup>2</sup> ) [11]
Root and leaf	Catechin	Proanthocyanidins		110.0 (PubChem CID 73160)

Database obtained from PubChem [11].

ligands and, consequently, as potential anti-ageing agents [55].

Anti-ageing ability is a recognised characteristic of antioxidant compounds, such as terpenes, fatty acids, and tocopherols [56–58]. Mo leaf extracts have been found to be terpene-rich ( $\alpha$ -phellandrene, *p*-cymene,  $\alpha$ -pinene, D-limonene and  $\beta$ -myrcene) [19,59], and a few works have shown the presence of fatty acids [14,18,19]. The unsaturated group was responsible for 32 % of the total relative concentration in Mo leaf extracts obtained by SFE-CO<sub>2</sub>. The linolenic acid reached more than 20 % (or 3.10 mg<sub>compound</sub>•g<sub>leaf</sub><sup>-1</sup>) [60]. The antioxidant potential of fatty acids has currently been evidenced in cosmetic emulsions [31]. This protection against skin oxidative stress is also promoted by tocopherol compounds with a high representativity in commercial cosmetics [56]. Mo leaf extracts were able to supply  $\alpha$ -tocopherol in significant quantities (22 % of relative concentration or 2.1 mg<sub>compound</sub>•g<sub>leaf</sub><sup>-1</sup>) to be identified as a potential cosmetic ingredient [19].

### 2.3. The twofold tannin activities from Mo root

A few works have reported the composition and bioactivity of Mo root extracts. Isothiocyanate alkaloids (e.g. morphine, moringin, and moringinine), flavonoids, saponins, tannins, triterpenoids, and steroids resume the chemical groups found in the sample material [10,19,27]. Among different properties, Mo root has shown a high antimicrobial effect against gram-positive (e.g. *Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (e.g. *Escherichia coli*), previously confirmed by comparing Mo root and leaf extracts [10].

Its antifungal activity was also noticed. Proanthocyanidins (e.g. catechin and gallic acid esters), a condensed tannin, were responsible for reducing strains of *Candida albicans* [9] and *Candida* spp. [61], a fungus usually present on the skin and mucous membranes such as the mouth, intestines, and vagina [10]. The compounds have also revealed a strong antioxidant activity, corroborated by the flavonoid content. Root extracts achieved a scavenger potential two-fold higher than Mo leaf extract, resulting in 70 times more antioxidant activity than the reference compound (ascorbic acid) [10].

Furthermore, 4-( $\alpha$ -L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate isothiocyanate alkaloids were associated with medicinal treatments for external wounds, tooth decay, and scurvy [2]. Isothiocyanate alkaloids have shown antibacterial and anti-inflammatory activities [54]. Antiulcer and cardiac stimulant properties have also been related to alkaloid molecules identified in Mo root extract [26].

Regarding anti-inflammatory activity, Mo root extract has been suggested to reduce acute and chronic inflammation conditions [62]. An inhibition higher than 80 % was observed in rats for both responses using an extract dose below the toxic concentration.

Finally, the dipeptides derivative aurantiamide acetate and 1,3-dibenzyl urea [63], as well as the deoxy-niazimicine aglycon compound [64], were found in Mo root extracts. These phytochemicals show anti-inflammatory and antimicrobial activity, respectively.

### 3. The potential of Mo compounds as cosmetic ingredients

Commercial cosmetic products containing Mo extracts have been disseminated to assess their benefits and economic potential. Skin care formulations have stated their ability to rejuvenate, nourish, and protect the skin, besides repairing, strengthening, and reducing hair loss [27]. Fig. 2a shows a progressive number of scientific publications correlating Mo extracts and cosmetic formulations from 1999 to 2023. According to the Scopus Online Database accessed in January 2024, 123 documents report the benefits of Mo phytochemicals as ingredients for skin or hair products [65]. However, very few studies provide valid information to support the bioactivity of Mo extracts introduced into topical products. Fig. 2b assesses the main keywords related to Mo and cosmetic subjects based on the co-occurrence network. Four clusters were formed from the

relevant terms. The highest occurrence number resulted in the larger interconnected circle, “*Moringa oleifera*”, which is mainly correlated to plant extract, phytochemistry, and bioactive compounds. Meanwhile, the cosmetic cluster emphasises oxidative stress, photoaging, skin care, sunscreen, and drug formulation.

Antioxidant activity forms the third cluster, which includes antimicrobial, anti-inflammatory, and antifungal activities. Finally, some of the plant parts (seed, leaf, and root) are highlighted and surrounded by their main compounds or group of compounds (e.g. vitamin, oleic and palmitic acids).

Fig. 2c shows the evolution of the studies over the last 25 years. Global analysis reveals that, predominantly, the association of “*Moringa oleifera*” and “cosmetic” took place around 2016, in which it is observed a progressive increase of terms related to bioactivity and commercial formulations (e.g. skin care and sunscreen). Green synthesis and phytochemistry keywords have shown higher relevance from 2020 to 2023.

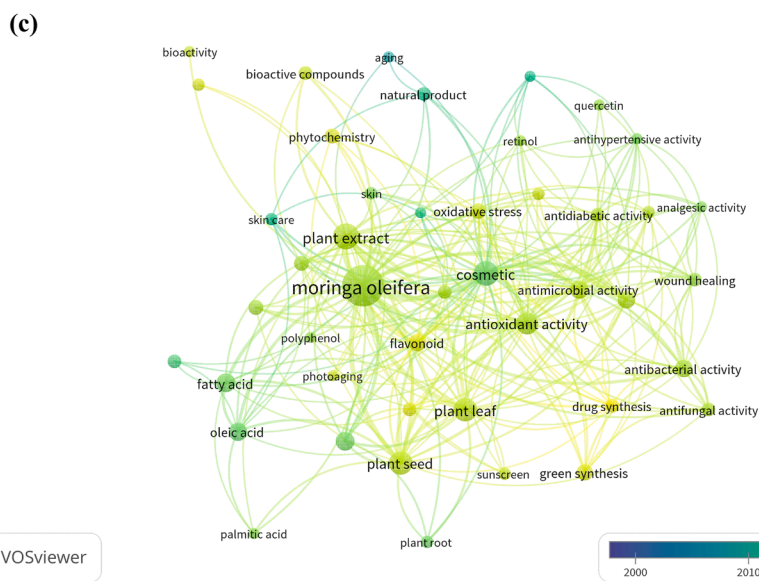
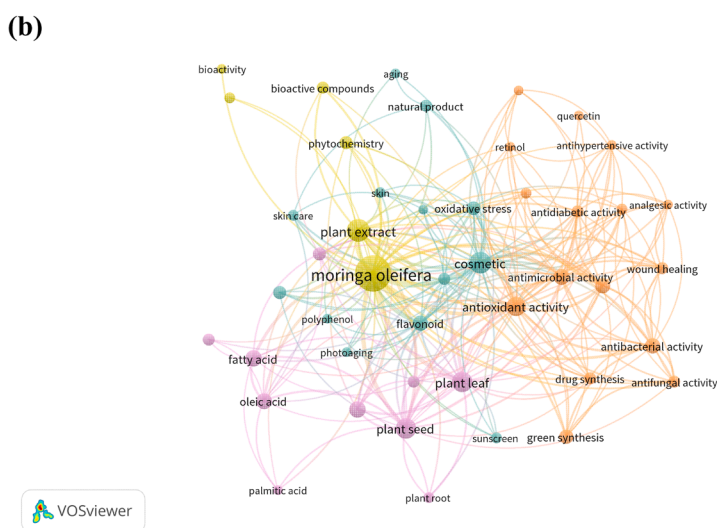
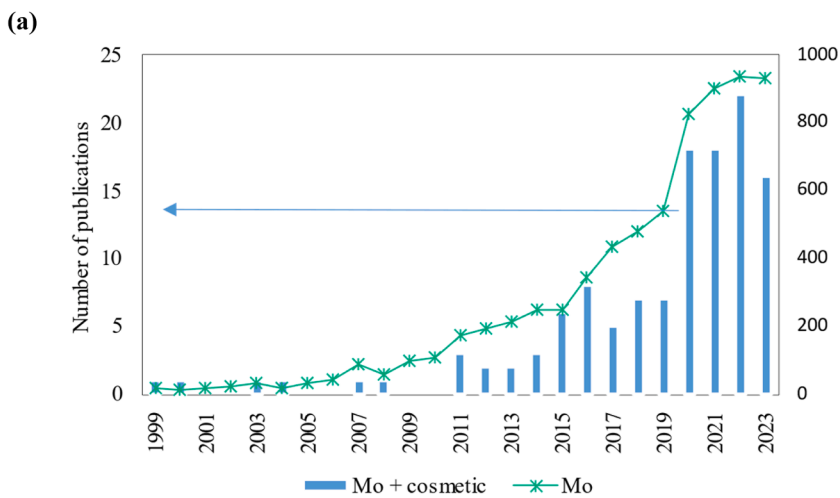
The data reflects recent research using Mo extract as a cosmetic ingredient. For example, Mo seed extract enhanced the skin hydration property of a cream. The effect was a response to the presence of  $\alpha$ -tocopherol, sterol, and fatty acid compounds, which also showed high antioxidant activity. The formulation reduced skin erythema without inducing skin irritation or affecting the melanin content [5]. Melanin content plays an important role in skin lightening. Its production results from the action of free radicals, which are observed through undesirable skin pigmentation. Antioxidants reduce oxidative stress by avoiding skin colour changes and preventing skin ageing [5]. These effects were emphasised using Mo seed extract incorporated into a cosmetic formulation. Melanin formation was reduced by 21.3 % after 6 days of topical application [66]. In addition, three formulations of Mo seed scrub cream were tested on white rats, and no acute dermal irritation was perceived over 48 hours. The emulsions remained stable at pH 6.8–7.3, with 3.5–5.5 % extract concentration [67].

As previously mentioned, flavonoids and phenolic acids are recognised as antioxidant bioactives. Their activity was an important factor in developing sunscreen and photoprotective Mo leaf-based formulations. Quercetin, rutin, kaempferol, ellagic acid, chlorogenic acid, and ferulic acid biomolecules have shown high antioxidant activity and anti-inflammatory activity, useful to prevent cellular damage and dermal inflammation promoted by ultraviolet light exposure [68]. The photoprotective effect was evidenced in sunscreen formulations composed of 2–4 % of Mo leaf extract. The protection activity against UV-B rays was higher than 50 %, explained by the high antioxidant activity, similar to those reported for *Origanum vulgare* and *Rosmarinus officinalis* species [68].

Mo leaves antioxidant activity has also justified the skin anti-ageing effect. A cream formulation containing Mo leaf extract was tested in eleven volunteers for three months [69]. A gradual decrease in roughness, scaliness, smoothness, and wrinkles has been noticed. Moreover, the increase in collagen and elastin structure explained the observed increase in skin hydration and elasticity. These results corroborate the recent study regarding the inhibition activity of Mo phenolic compounds against elastase, collagenase, and hyaluronidase enzymes [55].

Its use as a cosmetic ingredient includes body wash formulations. The scavenger potential of Mo leaf extracts has shown an effect against free radicals at concentrations up to 5 %. The extracts presented a rich composition of flavonoid (e.g. rutin, apigenin, quercetin, and kaempferol derivatives) and phenolic acids (e.g. caffeic acid, chlorogenic acid, gallic acid, and coumaroylquinic acid) [70]. The literature also reports the protection of skin keratinocytes, the major cell type of the human epidermis, due to the antioxidant activity of Mo stem extract. A topical treatment performed in rat epidermis promoted the inhibition of UVB-induced oxidative stress injury by using 6 % extract in a cream formulation. Rutin, luteolin, and quercetin were the main compounds responsible for the antioxidant activity [6].

The most recent update explores Mo leaf hydroethanolic and



**Fig. 2.** Scientific works reported in the period from 1999 to 2023, obtained from Scopus Online Database in January 2024 using as keywords *Moringa oleifera* (Mo) and cosmetic: (a) number of publications per year, (b) keywords co-occurrence network (4 clusters, selected keywords; number of occurrences 3), and (c) keywords co-occurrence network over time generated by VosViewer.

methanolic extracts and their antioxidant potential to be incorporated in cosmetic emulsions [71]. A final product containing 5 % of the extract was able to retain 50 % of the UVB radiation (or SPF 2) and has shown synergy towards Avobenzone - a commercial photo-unstable UVA filter - by avoiding its photodegradation and potentializing the UVAPF and SPF values, quantified after inducted irradiation.

Moreover, the anti-inflammatory potential of Mo leaves was also tested against atopic dermatitis. The extract reduced the keratinocytes-induced inflammation and increased the skin's epidermal and dermal thickness. A harmless activity regarding its cytotoxicity potential was obtained at a concentration range of 0.01–1.0 mg•mL<sup>-1</sup>, therefore suggested as a potential alternative for pharmacological and cosmetic formulations [7]. Phytochemicals such as quercetin and kaempferol glycosides derivatives, as well as isothiocyanates, have been related to the high anti-inflammatory activity of Mo leaf extracts [54,72].

The properties of compounds are slightly conditioned to their stability. These bioactives show reactivity under pH, light, heat, moisture, and oxygen variations, which may compromise their multiple functionalities during storage time [73]. The lipophilic fraction of Mo seed extracts improved the stability of cosmetic formulations. The oil shows a higher amount of saturated chains (e.g. behenic acid, stearic acid, and arachidic acid) and lesser polyunsaturated fatty acids (e.g. linoleic,  $\alpha$ -linolenic, and vaccenic acids) when compared with other oil sources [74], which reduces its reactivity. Also, technological systems, such as microencapsulation and microemulsions, have been used to protect and isolate target compounds from environmental stress by creating a physical barrier. The stabilisation methods can control the release of bioactive molecules, influenced by the systems' permeability [75].

Furthermore, the efficacy of transdermal drugs/bioactives depends on three factors: (i) their movement and release in the formulation; (ii) the bioactive release on the skin; and (iii) the bioactive solubility and permeability in the skin. The release behaviour was studied using bioactive quercetin extracted from Mo leaves and stabilised by gel-microemulsion. Stable formulations were obtained, and no significant difference was found between the emulsions for quercetin content. However, the viscosity significantly influenced the bioactive release rate. The highest formulation's viscosity resulted in a slower release rate [76]. Other potentialities of Mo extracts as dermatological and cosmetic ingredients have been reviewed recently [77], and reinforce the functional properties of Mo extracts (e.g., seed, leaf, and root).

Fig. 3 shows the number of patents issued from 1999 to 2023 (collected from the Free Patents Online (FPO) Database in January 2024) using the keywords “*Moringa oleifera* (Mo), cosmetic, and skin

care”. The individual keywords were searched as a claim (ACLM), while the combined ones had Mo as the claim and cosmetic or skin care included in the abstract (ABST). The numbers consider data from United States (US) Patents and Documents, European Union (EP), Abstracts of Japan, World Intellectual Property Organization (WIPO/PCT), and German patents. Fig. 3 shows a trend at the beginning of the last decade (2010), in which cosmetics represent 50 % of the uses of Mo. A plateau of the uses of Mo remains from 2016 to 2019 – around 50 patents – in agreement with documents referring to cosmetic/skin care. From 2020, a considerable decrease in publications took place, possibly correlated to the COVID-19 pandemic.

Table 2 summarises selected inventions according to their patent assignee and function description. Among the active ingredients introduced into cosmetic formulations, at least one is related to Mo sources, usually composing skin and hair products.

#### 4. Conventional and alternative extraction processes in Mo extract composition

Recovering bioactive molecules from natural sources requires efficient and safe processes. Various methods can be used to extract the respective phytochemicals, resulting in different mixtures of active and inactive compounds. Organic solvent-based methods, such as Soxhlet (SOX), maceration (MAC), ultrasound-assisted (UAE) [42,55], and microwave-assisted (MAE) [12,31] are the most common. Hydro-distillation (HD), steam distillation (SD) [44], pressurized hot water extraction (PHWE) [52], pressurized liquid extraction (PLE) [96], mechanical cold pressing (CP) [43], carbon dioxide-expanded ethanol (CXE) [14], and enzymatic techniques [97] have also been used to perform Mo extractions. Some of these methodologies show negative effects regarding the extracts' chemical stability and compounds' bioactivity, mainly due to the presence of thermolabile compounds. Extraction time, use of toxic solvents, solvent residues in the final product, and negative environmental impact have been listed as the main drawbacks concerning conventional technologies, increasing the need for green solutions [13].

These disadvantages notwithstanding, organic-solvent extractions (e.g. SOX, MAC, UAE, and MAE) require minimal effort to obtain the extracts from either liquid or solid samples [39]. Usually, the procedures are performed using *n*-hexane [13,41], petroleum ether [97], acetone [98], ethyl acetate [99], ethanol [100], and methanol [45]. The efficiency of these extraction methodologies is conditioned to the affinity of the solvent and the compounds to be extracted from the raw material,

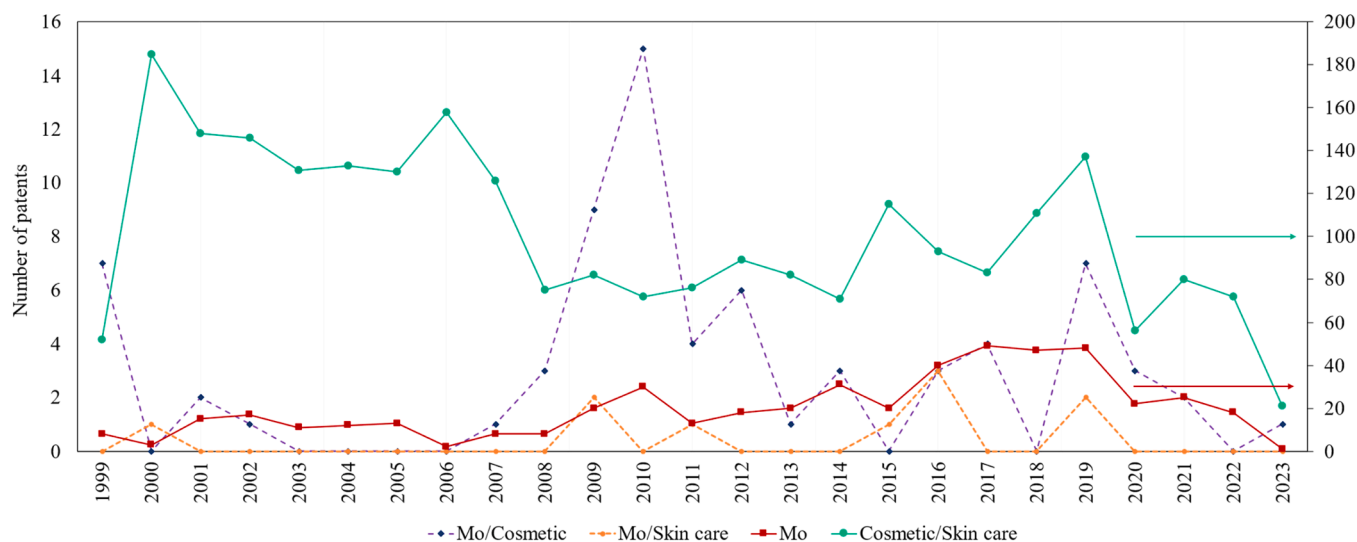


Fig. 3. Number of patents published in the period from 1999 to 2023 (obtained on Free Patents Online Database (FPO), January 2024; Keywords: *Moringa oleifera* (Mo), cosmetic, skin care). \*full lines correspond to the secondary axis.

**Table 2**

Description of selected inventions registered from 1999 to 2023, using the keywords "Moringa oleifera (Mo), cosmetic, and skin care", assessed in Free Patents Online (FPO) Database in January 2024.

Patent assignee	Summary of invention	Plant part
Henkel KGaA (Dusseldorf, DE) [78] United States Patent 7553338 International Flute Technologies LTD (Gilbert, AZ) [79] United States Patent 6667047	Cosmetically colour-modifying agents and care effect (at least one protein) from <b>Mo seed</b> . High slip value of cosmetic composition of long-chains oil from <b>Mo seed</b> , with methylene interrupted unsaturation of less than 1 %. Formulation supplemental additive (malic acid, kojic acid or ascorbic acid) to use as skin- and hair-care products. Topical product composed of at least one protein extract of the Mo seed for hydrating, repairing, producing anti-wrinkle effects and/or producing anti-pollution effects for the skin, lips, nails and/or hair of a person.	Seed
Laboratoires Serobiologiques (Pulnoy, FR) [80] United States Patent 6500470	Topical product composed of at least one protein extract of the Mo seed for hydrating, repairing, producing anti-wrinkle effects and/or producing anti-pollution effects for the skin, lips, nails and/or hair of a person.	
CLARITY COSMETICS INC. (Potomac, MD, US) [81] United States Patent 10159637	Non-comedogenic and/or non-acneogenic hair care formulations based on <b>Mo seed</b> extracts for the cleaning, conditioning, and/or styling of the hair of a user without causing or promoting irritation or acne on the skin of the user. Such a formulation may also help resolve skin irritation, breakouts, and/or acne.	
FABRE PIERRE DERMO COSMETIQUE (FR) MANDEAU ANNE (FR) DUPLAN HELENE (FR) [82] WIPO Patent Application WO/2010/149895	The invention uses <b>Mo seed oil</b> (triglycerides, fatty acids, polar lipids, and polyphenols) to enrich a cosmetic and/or dermatological formulation.	
BAKEL SRL (IT) [83] WIPO Patent Application WO/2017/055943	Cosmetic composition for healing and treating the skin. The formula consists exclusively of compounds functional ( <b>including Mo oil seed</b> ) for the skin and/or compounds functional both for the skin and also the structure of the formula.	
UNILEVER IP HOLDINGS B V (NL) UNILEVER GLOBAL IP LTD (GB) CONOPCO INC DBA UNILEVER (US) [84] WIPO Patent Application WO/2022/135882	Personal care composition that provides - alone or combined - anti-inflammatory, anti-ageing, and anti-acne benefits, composed by <b>Mo extract</b> (preferentially from seed), and a hydroxy fat acid in proportions 1:1-75:1.	
UNILEVER IP HOLDINGS B V (NL) UNILEVER GLOBAL IP LTD (GB) CONOPCO INC DBA UNILEVER (US) [85] WIPO Patent Application WO/2022/135883	Personal care composed by <b>Mo extract</b> (from seed) genus Theobroma, and fractions of vitamin B3. The formulation claims to alleviate, inhibit and/or reduce at least one symptom selected from inflammation, gingivitis, periodontitis, dandruff and acne.	Seed
CLARITY COSMETICS INC. (Potomac, MD, US) [86] United States Patent 10813872	Non-comedogenic and/or non-acneogenic hair care formulations ( <b>including Mo oil seed</b> ) for the cleaning, conditioning, reducing inflammation of hair follicles and/or styling of the hair. No causing or promoting irritation or acne on the skin of the user. Such a formulation may also help resolve skin irritation, breakouts, and/or acne.	
Access Business Group International LLC (Ada, MI, US) [87] United States Patent 10383806	Cosmetic compositions for topical use that have anti-aging, antioxidant, anti-irritant, anti-inflammatory, and/or aesthetic improvement properties obtained from natural extracts ( <b>including Mo seed extract</b> ).	

**Table 2 (continued)**

Patent assignee	Summary of invention	Plant part
BAKEL SRL (Udine, IT) [88] United States Patent 11013681	A cosmetic composition ( <b>including Mo seed extract</b> ) for healing and treating the skin, with functional compounds for the skin and/or the structure of the formula. All formulation is comprised by active compounds and completely absorbed by the skin.	
Wehrli Janet M. (Omaha, NE, US) [89] United States Patent 10092488	A system to neutralize acids in dental plaque biofilm, inhibit plaque from adhering to teeth and gums, encourage removal of dental plaque, and form a barrier between the teeth and gums and the oral environment. A formulation comprising by selected oil groups ( <b>including ben oil from Mo seed</b> ).	
SHAMROC, INC. (Carlsbad, CA, US) [90] United States Patent 9949919	Emollient and an anti-irritant formulation comprised by an oil-in-water emulsion ( <b>including Mo seed extract</b> ). The method and composition decrease the irritancy of hydroquinone-containing topical formulations.	
The Procter & Gamble Company (Cincinnati, OH, US) [91] United States Patent 9358263	Personal care compositions with one or more botanical extracts ( <b>including Mo extract from leaves</b> ) and/or skin care actives to provide prophylactic and therapeutic regulation of keratinous tissue conditions. The compositions are useful for improving skin tone and texture, e.g. lightening skin, reducing the appearance of hyperpigmentation, and reducing the appearance of wrinkles.	Leaf
STEMTECH INTERNATIONAL, INC. (San Clemente, CA, US) [92] United States Patent 20140227363	Skin care composition containing combination of natural ingredients ( <b>including Mo leaf extract</b> 0-1.5 g•50 mL <sup>-1</sup> ) that exhibits synergistic effects to improve skin moisture, wrinkle reduction, and elasticity. The individual compounds improve collagen production, fibroblast proliferation, antioxidant protection and free radical inhibition.	
Y&B Mother's Choice Ltd. (Jerusalem, IL) [93] United States Patent 10434058	The formulations (mixture of natural compounds, <b>including MO oil</b> ) are suitable for use on hair and skin, for delivering cosmetic or therapeutic actives to the skin for providing cleansing, moisturizing, minimizing, or treating skin imperfections, reducing skin oiliness, providing fragrance to the hair or skin and further provide the benefit of reliving skin dryness and signs of aging.	N/A
IVY COSMETICS CORP [94] Japanese Patent JP2011101898A	A skin care preparation for antiaging that activates fibroblast in dermis of the skin and inhibits onset or progress of skin aging symptoms such as wrinkles and flabbiness accompanied by transition with age. The composition is whey protein basis and <b>includes Mo extract</b> .	

which is highly influenced by their polarity.

For example, a range of solvents (*n*-hexane, ethyl acetate, and ethanol) was used in MAC extraction of Mo seed [99]. The results revealed a higher extraction yield by reducing the solvent polarity. Therefore, the experiment performed with *n*-hexane showed an increased efficiency regarding the total extract recovered, followed by ethyl acetate and ethanol. This high value is an effect of the well-known fatty acid composition (non-polar chains) of Mo seed combined with the

absence of OH groups in the *n*-hexane solvent. In addition, the dipole moment of polar solvents can lead to lipids' hydrolysis process. However, *n*-hexane is recognised as a non-selective solvent, responsible for extracting non-volatile pigments and undesirable waxed compounds [13].

Polar compounds of Mo leaves were also extracted using UAE. Varying the water:ethanol ratio, that is, increasing the solvent's polarity, improves both extraction yield and total phenolic content due to the higher compounds' solubility [101]. The compounds' solubility is directly correlated to their molecular polarity. For example, polar compounds such as organic acids (e.g. gluconic, quinic, malic, and citric acids), nucleosides, and phenolic acids derivative (e.g. vanillin glucoside, caffeoylquinic, feruloylquinic, coumaroylquinic acid, and their respective isomers) were more readily obtained than flavonoids (e.g. quercetin-3-*O*-glucoside, kaempferol 3-*O*-glucoside, apigenin glucoside, and their isomers).

Experiments combining PHWE methodology and Mo leaf samples [14] have shown a lower extraction affinity to low-polar organic acids and lignans (e.g. isolariciresinol glycoside isomers) compared to high-polar compounds. A second study corroborates to the importance of the solvent's polarity on the extraction yield, emphasising the temperature influences. By raising the temperature, the solvent's polarity reduces due to the weakening hydrogen bonds, which improved the extraction of kaempferol and quercetin flavonoids. However, above 100 °C, the hydrolysis process takes place and significant losses were observed in the kaempferol extraction [52]. Table 1 shows some of the molecular structures of target compounds extracted from the Mo tree and their respective polar surface area.

High temperatures have often been referred as a limiting factor for the extraction of bioactives. This effect was observed in organic solvent extraction, resulting in a lower fraction of antioxidant compounds and, consequently, lower oxidation stability. The thermal decomposition of flavonoids, phenolic acids, and unsaturated fatty acids reduced the chemical quality of extracts obtained by SOX [13]. High temperatures and bioactive decomposition have also been reported for the HD method, in which the sample material must be submitted to a temperature above the boiling point of water to carry the oil. Furthermore, HD is restricted to volatile compounds and does not allow the extraction of phenolic compounds [44].

Therefore, different alternatives have been proposed to avoid the degradation processes. CP technology has been indicated as an option for obtaining high-quality products from Mo seed. The methodology was compared with MAC extractions using *n*-hexane and chloroform-methanol, respectively, and it has shown higher stability [43].

Despite the effort to maximise the stability of phytochemical compounds extracted by conventional methods from the Mo plant [102], the main drawbacks, such as thermal degradation and the use of organic solvents, prevail. Thus, other methods are required to extract bioactive molecules safely and widely for food, pharmaceutical, and cosmetic applications.

Supercritical carbon dioxide extraction (SFE-CO<sub>2</sub>) has been suggested as an alternative technology to overcome these issues [74] and it is further discussed below. Table 3 summarises recent studies performed with the Mo tree, including the extraction method, yield, declared bioactivities, and chemical composition.

## 5. Selectivity and stability of Mo extracts obtained by SFE-CO<sub>2</sub>

Innovative extraction processes have been described as "green technologies" for providing harmless alternatives regarding the solvent, ensuring maximum solvent recovery and reuse, reducing operation costs, and reducing environmental impact [112]. The literature explains the operation of alternative extraction methods and their main characteristics [113], including details of SFE-CO<sub>2</sub> equipment previously used to obtain Mo extracts from seed, leaf, and root [19]. SFE technology plays an important role in this sense. This extraction methodology uses a

non-toxic solvent, offers high efficiency, and shows environmentally friendly features with flexible operation parameters [114], besides stable thermal conditions to obtain solvent-free extracts with high selectivity [101].

The most common solvent used in the SFE methodology is carbon dioxide (CO<sub>2</sub>), which is Generally Recognised as Safe (GRAS) by the Food and Drug Administration (FDA) and the European Commission [114,115]. Due to the low viscosity, high diffusivity, and consequently high-density power, CO<sub>2</sub> in its supercritical state can readily penetrate the sample material by an accelerated mass transfer rate [95]. The supercritical phase is achieved by tuning the pressure (*P*) and temperature (*T*) above the critical point (*P*=74 bar and *T*=32 °C). Changes in *P* and *T* influence the solvent density and solubility and can be used as a strategic factor for obtaining a target composition of extracts. Moreover, fractionating of the extracts can be achieved by controlling the system depressurisation, offering a significant advancement in the separation of unwanted compounds and the concentration of desired active compounds [17].

However, CO<sub>2</sub> has a non-polar profile. The addition to a co-solvent can ensure its polar affinity and improve the extraction of dipole-charged compounds. Ethanol is often used as a co-solvent in SFE-CO<sub>2</sub> extractions. The synergy between both solvents decreases the interfacial tension on the sample surface, improving the diffusivity of polar compounds [96]. An alternative strategy aiming at target compounds and specific bioactivities involves downstream processing. This approach has been previously implemented by introducing other techniques such as CXE and PHWE following SFE-CO<sub>2</sub> extraction, leading to higher yields of phenolic content and enhanced antioxidant activity [14].

In turn, the diffusivity power also results from the compound's vapour pressure and its interaction with the solvents, which is noticed by the solubility degree [42]. The molecule's solubility has been evaluated by optimisation studies varying the pressure, temperature, particle size, solvent flow rate, and residence time in the extraction cell [105,106]. A detailed discussion regarding the influence of SFE-CO<sub>2</sub> parameters on the extraction of bioactives from the Mo tree is presented in the following sections. Mainly, the studies have focused on seed samples. However, some considerations are explored for Mo leaf extractions.

### 5.1. Pressure and temperature

Pressure and temperature have been mentioned as the most influential parameters on SFE-CO<sub>2</sub> technology and, thus, often recognised as the main subject in optimisation studies of Mo seeds [104,105,116] and leaves [17,18]. The extraction process is based on the mass transfer phenomena dominated by the diffusive mechanism, in which the solvent is solubilised into the sample particle. Fig. 4 illustrates the diffusion of supercritical CO<sub>2</sub> into the particle matrix and its extraction phases to obtain the compound mixture. The solvent power solubilisation can be improved by varying the pressure and temperature and reflected on the total extraction yield or extraction yield of a target compound. The extraction efficiency results from the interplay between solvent density and compound solubility. At a constant temperature, increasing the pressure boosts solvent density, enhancing compound solubility. However, when the temperature rises, deviations occur; below 300 bar, the solvent density may decrease, whereas above 300 bar, it tends to increase [17].

Both pressure and temperature parameters significantly affect the initial extraction period, in which (Fig. 4a-i) the solvent penetrates the superficial particle layer by a diffusion process [117]. This period is called the constant-extraction rate and is responsible for the faster mass transfer. Previous studies identified the initial extraction period occurrence of up to 120 min [13] and 20 minutes [14] for Mo seed and leaf samples, respectively. The extraction process also encompasses the compounds' dissolution and separation from the matrix (Fig. 4a-ii), the diffusion of the extracted compounds out of the particle surface (Fig. 4a-iii), and the recovery of the extract from the sample during the

Table 3

Summary of recent studies regarding SFE-CO<sub>2</sub> technology and conventional extraction methodologies, their respective extraction yield, total phenolic, flavonoid and tocopherol content, and main compounds identified in Mo seed, leaf, and root extracts.

Plant part	Reference	Extraction method and extraction yield	Content	Main compounds
Seed	Effect of supercritical carbon dioxide conditions on extraction of food phytochemical constituents from <i>Moringa oleifera</i> . Lam seed kernels [103]	SFE-CO <sub>2</sub> -EtOH (35.3 %) and SOX (17.1 %)	TPC: 41.8–44.7 TFC: 14.8–18.2	-
	Ultrahigh-pressure supercritical fluid extraction and chromatography of <i>Moringa oleifera</i> and <i>Moringa peregrina</i> seed lipids [42]	SFE-CO <sub>2</sub> (238–396 mg/g) and UAE (251 mg/g)	-	Oleic acid, linolenic acid, behenic acid, campesterol, and stigmasterol
	Optimization of supercritical extraction process for <i>Moringa</i> (PKM–1) seed kernel oil [104]	SFE-CO <sub>2</sub> -EtOH (31.87–37.76 g/100 g) and SOX (29.12 g/100 g)	-	-
	Microwave pretreatment of <i>Moringa oleifera</i> seed: Effect on oil obtained by pilot-scale supercritical carbon dioxide extraction and Soxhlet apparatus [13]	SFE-CO <sub>2</sub> (30.7 %), SOX (36.44 %) MAE-SFE-CO <sub>2</sub> (30.63–35.28 %), and MAE-SOX (27.4–40.0 %)	TTC: 400.0–700.0	Oleic acid, behenic acid, stearic acid, and $\alpha$ -tocopherol
	Extraction and characterization of oil from <i>Moringa oleifera</i> using supercritical CO <sub>2</sub> and traditional solvents [100]	SFE-CO <sub>2</sub> (<0.5–2.0 %) and SOX (30–35 %)	-	Oleic acid, palmitic acid, and stearic acid
	Extraction of oil from <i>Moringa oleifera</i> kernels using supercritical carbon dioxide with ethanol for pretreatment: Optimization of the extraction process [105]	SFE-CO <sub>2</sub> -EtOH (31.9–37.8 %) and SOX (37.5 %)	-	Oleic acid, behenic acid, stearic acid, and arachidic acid
	Experimental Modeling and Simulation of Supercritical Fluid Extraction of <i>Moringa oleifera</i> Seed Oil by Carbon Dioxide [106]	SFE-CO <sub>2</sub> -EtOH (-) and SOX (35.8 %)	-	-
	<i>Moringa oleifera</i> oil: Studies of characterization and biodiesel production [107]	SOX (39.0 %)	-	Oleic acid, palmitic acid, behenic acid, arachidic acid, and stearic acid
	Process optimization of solvent extraction of seed oil from <i>Moringa oleifera</i> : An appraisal of quantitative and qualitative process variables on oil quality using D-optimal design [99]	SOX (2.2–42.0 %)	-	Oleic acid and behenic acid
	Analytical characterization of <i>Moringa oleifera</i> seed oil grown in temperate regions of Pakistan [41]	SOX (40.39 %)	TTC: 41.0–161.3	Oleic acid, palmitic acid, stearic acid, behenic acid, arachidic acid, and $\alpha$ , $\gamma$ , $\delta$ -tocopherol
	The application of ultrasound and microwave to increase oil extraction from <i>Moringa oleifera</i> seeds [108]	SOX (39.2 %), UAE (35.8 %) and MAE (36.9 %)	-	Oleic acid, palmitic acid, and behenic acid
	Some physico-chemical properties of <i>Moringa oleifera</i> seed oil extracted using solvent and aqueous enzymatic methods [97]	SOX (30.8 %) and enzymatic (22.6 %)	-	Oleic acid, palmitic acid, stearic acid, and behenic acid
	Characterization of <i>Moringa oleifera</i> seed oil variety “Periyakulam 1” [43]	CP (25.1–41.4 %)	TCC: 20.6–216.7	Oleic acid, behenic acid, palmitic acid, $\beta$ -sitosterol, and $\alpha$ , $\beta$ , $\gamma$ -tocopherol
	Seed and root	Phytochemistry and antifungal activity of root and seed extracts of <i>Moringa oleifera</i> [10]	MAC	-
<i>Moringa oleifera</i> L. screening: SFE-CO <sub>2</sub> optimisation and chemical composition of seed, leaf, and root extracts as potential cosmetic ingredients [19]		SFE-CO <sub>2</sub> (0.06–3.38 %)	-	Oleic acid, $\gamma$ -sitosterol, linolenic acid, nonacosane, and $\alpha$ -tocopherol
Leaves and seed	Supercritical fluid extracts of <i>Moringa oleifera</i> and their unsaturated fatty acid components inhibit biofilm formation by <i>Staphylococcus aureus</i> [45]	MAE-SFE-CO <sub>2</sub> -EtOH (3.4–9.1 %) and MAE-MAC (0.6–72.3 %)	-	cis–11-Eicosenoic acid, palmitic acid, linoleic acid, stearic, oleic acid, multiflorin B, quercetin–3-O-glucoside, and kaempferol–3-O-glucoside
	Volatile and non-volatile chemical constituents of <i>Moringa oleifera</i> Lam., Moringaceae [109]	HD and MAC	-	Thymol, phytol, hexadecanoic acid, hexadecyl acetate, tetracosane, docosane, tetracosane, and octacosane
	Nutritional and phytochemical profiles and biological activities of <i>Moringa oleifera</i> Lam. edible parts from Guinea-Bissau (West Africa) [110]	DC	TPC: 0.1–14.7 TFC: n.d. to 10.3 TTC: 27.0–210.0	Oleic acid, behenic acid, stearic acid, $\alpha$ , $\gamma$ -tocopherol, quercetin derivatives, and kaempferol derivatives
	An experimental investigation into the solubility of <i>Moringa oleifera</i> oil in supercritical carbon dioxide [111]	SFE-CO <sub>2</sub> (~35.0 %)	-	Oleic acid, behenic acid, and cis-vaccenic acid
Leaves	Extraction of compounds from <i>Moringa oleifera</i> leaves using supercritical CO <sub>2</sub> plus ethanol as a cosolvent [18]	SFE-CO <sub>2</sub> -EtOH (1.5–6.1 %) and SOX (25.2 %)	TPC: 18.0–39.0	1-triacontanol, nonacosane, heptacosane, gallic acid, vanillic acid, caffeic acid, p-coumaric acid, catechin, epicatechin, and quercetin
	Green downstream processing using supercritical carbon dioxide, CO <sub>2</sub> -expanded ethanol and pressurized hot water extractions for recovering bioactive compounds from <i>Moringa oleifera</i> leaves [14]	SFE-CO <sub>2</sub> (1.4–3.1 %) and PHWE	TPC: 20.3–62.4 TFC: 3.8–9.6	Tricontane, nonacosane, ethyl octadecanoate, octadecanal, kaempferol–3-O-glucoside, and quercetin–3-O-glucoside
	Evaluation of the effect of different co-solvent mixtures on the supercritical CO <sub>2</sub> extraction of the phenolic compounds present in <i>Moringa oleifera</i> Lam. leaves [53]	SFE-CO <sub>2</sub> -EtOH and SFE-CO <sub>2</sub> -H <sub>2</sub> O	TFC: 3.0–10.0	Apigenin glucoside isomer 1 and 2, quercetin 3-O-glucoside, kaempferol 3-O-glucoside, and kaempferol malonyl glucoside
	Chemical composition and biological activity of the essential oil from leaves of <i>Moringa oleifera</i> Lam. cultivated in Mozambique [44]	HD (0.05 %)	-	Hexacosane, pentacosane, heptacosane, luteolin and, quercetin–3-O-glucoside

(continued on next page)

Table 3 (continued)

Plant part	Reference	Extraction method and extraction yield	Content	Main compounds
	Development of pressurised hot water extraction (PHWE) for essential compounds from <i>Moringa oleifera</i> leaf extracts [52]	PHWE	TFC: 4.7–7.0	-
	Comparison of Volatile Profile of <i>Moringa oleifera</i> Leaves from Rwanda and China Using HS-SPME [12]	MAE	TPC: 24.6–30.0 TFC: 39.0–52.8 TPC: 10.93	Longifolene, geranyl acetone, $\beta$ -ionene, acetic, hexanoic acid, and hexadecanoic acid
	<i>Moringa</i> leaf: An innovative source of antioxidative phenolics for cosmeceutical products [31]	MAE (34.6 %)	TPC: 20.3–62.4 TFC: 3.8–9.6	-
	Optimization of extraction method to obtain a phenolic compounds-rich extract from <i>Moringa oleifera</i> Lam leaves [101]	MAC and UAE	TPC: 20.3–62.4 TFC: 3.8–9.6	Tricortane, ethyl octadecanoate, nonacosane, octadecanal, quercetin–3-O-glucoside, kampferol 3-O-glucoside, quinic acid, multiflorin B and, caffeoylquinic acid
	Potential anti-aging components from <i>Moringa oleifera</i> leaves explored by affinity ultrafiltration with multiple drug targets [55]	UAE		Caffeoylquinic acid derivatives, quinic acid, quercetin derivatives, kaempferol derivatives, vicenin–2, apigenin derivatives, and N-fructosyl pyroglutamate
	Detailed chemical composition and functional properties of <i>Ammodaucus leucotrichus</i> Cross. & Dur. and <i>Moringa oleifera</i> Lamarck [72]	DC	TPC: 29.0–43.5 TFC: 17.4–30.0 TTC: 45.0	Oleic acid, behenic acid, $\alpha$ -linolenic acid, palmitic acid, quercetin–3-O-glucoside, and $\alpha$ , $\gamma$ -tocopherol
	Multifunctional profiling of <i>Moringa oleifera</i> leaf extracts for topical application: a comparative study of different collection time [71]	MAC	TPC: 52.0–83.0	Chlorogenic acid, ellagic acid, ferulic acid, rutin, and quercetin

TPC: total phenolic content ( $\text{mg GAE} \cdot \text{g}^{-1}$ ); TFC: total flavonoid content ( $\text{mg RE} \cdot \text{g}^{-1}$ ); TTC: total tocopherol content ( $\text{mg} \cdot \text{kg}^{-1}$ ); SFE- $\text{CO}_2$ : supercritical  $\text{CO}_2$  extraction; SOX: Soxhlet; UAE: ultrasonic assisted extraction (UAE); MAE: microwave-assisted extraction; CP: cold press extraction; MAC: maceration extraction; HD: hydro-distillation extraction; PHWE: pressurized hot water extraction; DC: decoction extraction; EtOH: ethanol; n.d.: no detected.

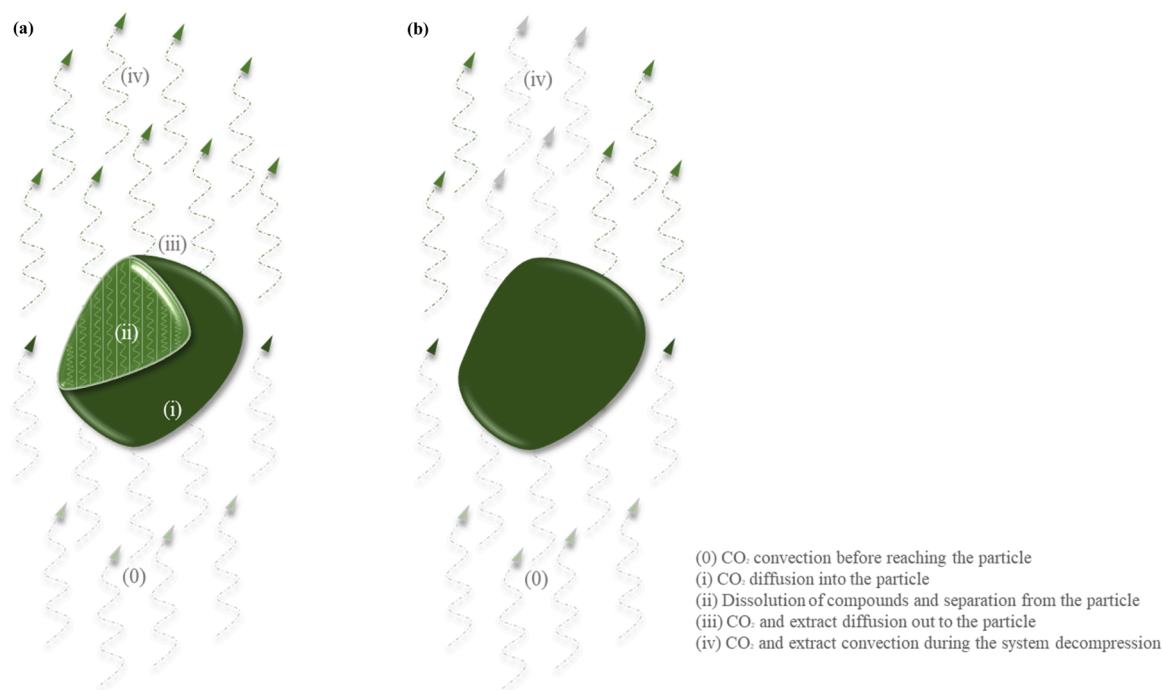


Fig. 4. Scheme of the supercritical  $\text{CO}_2$  convection and diffusion into and out of the particle, respectively. (a) extraction phases to obtain the mixture of the compounds, (b) bypass-like process; particle creates a thick shell and is surrounded by supercritical  $\text{CO}_2$ .

decompression by convective process (Fig. 4a-iv) [113,117].

The recurring effect of pressure and temperature has been noticed throughout the extraction time, which makes the SFE- $\text{CO}_2$  technology a remarkable strategy to ensure the selectivity of the extracts. A wide variability of extraction conditions for Mo seed has been reported, resulting in different compositions of extracts. For example, increased phenol content was correlated to pressure above 150 bar due to a higher solvent density. However, the extraction efficiency was limited by

raising the temperature to 50 °C [103]. Usually, high temperature has a negative influence on the extraction of thermolabile compounds, explained by the degradation processes adding to the decrease in  $\text{CO}_2$  density and solvation power [117]. However, the temperature shows an inverse behaviour when combined with high pressure. This phenomenon is called crossover pressure and results from enhancing the vapour pressure of the analytes and their solubility by increasing the temperature at a pressure above 300 bar [118]. Furthermore, increased lipid

fraction is an effect of high CO<sub>2</sub> densities [42]. The crossover effect has been reported for Mo seed extractions performed at 300–500 bar and 60–100 °C [95], 200–500 bar and 60–100 °C [111], and 400–800 bar and 40–70 °C [42].

However, a higher extraction yield may not represent higher bioactivity. Thermolabile compounds obtained from Mo seed, such as tocopherols, carotenoids, and phenolics, have shown a higher activity if extracted at low temperatures. An SFE-CO<sub>2</sub> experiment carried out at 300 bar and 40 °C has resulted in higher oxidation stability and higher extraction yield of the target compounds compared to Soxhlet extracts [13]. Different studies have shown that an increase in the total extraction yield does not necessarily result in increasing bioactive extraction. Lowering the pressure from 350 to 150 bar, a higher content of  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and oleic acid was obtained from Mo seed, although the highest pressure resulted in the highest extraction yield [116]. Operating at 195 bar, Kessler et al. [19] obtained a selectivity of  $\alpha$ -tocopherol up to 21 % for leaf extracts compared to 17 % using 273 bar at the same temperature. Once again, the higher pressure has resulted in a 27 % higher extraction yield.

Considerations regarding the target compound to be extracted and its bioavailability on the sample matrix are essential to increase the process selectivity. An extraction performed with Mo leaf identified the highest extraction yield working at 500 bar and 60 °C [17]. However, in terms of the relative composition of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol, the compounds performed better at 400 bar and 40 °C and 300 bar and 80 °C, respectively. Furthermore, a mild extraction condition was applied to Mo leaves to study the co-solvent influence on the extraction yield and chemical composition [18]. At 250 bar and 35 °C, the co-solvent did not help increase the extraction yield or improve the relative composition of the tocopherol content. However, a richer phenolic and organic acid composition was observed during the extraction due to higher solvent polarity, also indicated by the bioactivity results. Further review of the co-solvent effect is given below.

In turn, the lack of flavonoid content was mentioned for extracts obtained from Mo seed and leaf samples at 300 bar and 60 °C [45]. The SFE-CO<sub>2</sub> products indicated a relevant relative composition of fatty acids, mainly oleic acid (C<sub>18:1</sub>) and *cis*-11-eicosenoic acid (C<sub>20:1</sub>), respectively, responsible for the antibiofilm and antioxidant activities of Mo extracts. Usually, at high pressure, there is a tendency to obtain a higher fraction of lipophilic molecules, especially those with longer and unsaturated chains [42].

To summarise, ideal SFE-CO<sub>2</sub> conditions regarding the highest extraction yield of selected compounds have been reported in the literature: for Mo seeds at 40 °C and 200 bar [104], 50 °C and 200 bar [103], 40 °C and 300 bar [13], 45 °C and 290 bar [105], 57 °C and 800 bar [42]; and for Mo leaves at 50 °C and 150 bar [14], 60 °C and 200 bar [18], and 60 °C and 500 bar [17], while a SFE-CO<sub>2</sub> optimisation had reported experiments at 41–69 °C and 117–273 bar for Mo root samples [19].

## 5.2. Co-solvent

Supercritical CO<sub>2</sub> offers various advantages to extracting compounds from natural sources, mainly because the thermolabile compounds remain with fresh characteristics [60,95,113]. The non-polar profile of CO<sub>2</sub> favours the extraction of non-polar molecules, such as sterol and fatty acids [17,45,88]. To improve the polar solvation power, ethanol can be used as a co-solvent to extract flavonoids, phenolic, and organic acid compounds, for example [14,18,105].

The solvent modifier can also enhance the extraction of polar lipids (e.g. phosphatidylinositol and phosphatidylserine) by increasing the dipole-dipole and hydrogen bond interactions between ethanol and the extract's polar functional groups [42]. These effects are correlated to a higher solvent density. The polar and non-polar compounds' solubility has increased 1.85 times [106] and 11.11 times [105] in Mo seed extractions using 10 % ethanol.

Ethanol influence includes the enhancement of the raw material's

surface area due to the solid-solid interaction, improving solvent accessibility [18]. Mo leaf SFE-CO<sub>2</sub> extraction at 150 bar, 50 °C, and 50 % ethanol revealed a two-fold higher extraction yield than the experiment performed without co-solvent at the same pressure and temperature [14]. This effect was not observed for extractions carried out at 250 bar, 35 °C, and 0–50 % of ethanol [18]. However, due to the higher solvent polarity, ethanol favoured obtaining phenolic acids from Mo leaves.

Low co-solvent concentrations have not affected the extraction of polar compounds; 5 % ethanol was used in SFE-CO<sub>2</sub> of Mo seed and leaves, resulting in poor flavonoid content. Nonetheless, an improvement in the unsaturated fatty acid fraction was identified [45]. The claim of polar compound's improvement has also been refuted on the SFE-CO<sub>2</sub>-EtOH extractions of Mo leaves, in which no significant amounts of flavonoids were obtained. The dissolution of polar compounds was, however, possible by using 50 % water as a co-solvent [53]. The contribution of the water towards supercritical CO<sub>2</sub> extractions has shown an enhancement of the global yield by increasing the solubility of a wider number of compounds, which, however, resulted in losses of selectivity [119]. To obtain a more selective flavonoid extract, studies have recommended small fractions of EtOH and water combined as SFE-CO<sub>2</sub> modifiers [119,120].

## 5.3. Particle size

SFE-CO<sub>2</sub> extraction optimisation using the Mo plant parts has been performed. As previously discussed, pressure, temperature, and co-solvent are the main contributors to total extraction yield and compound selectivity [14,18,105,106,116,117]. Some studies have also considered the particle size influence, mostly related to Mo seed samples [105,106,116,117].

The highest effect of the particle size can be observed during the diffusion phase. The phenomenon holds the first to third extraction stages, as exemplified in Fig. 4. Reduced particle size may offer a shorter diffusion path, higher surface area, and lower intraparticle resistance, resulting in a higher extraction yield [117]. Extraction yields varied between 25 % and 40 % for particle sizes between 2.0 and 0.5 mm for Mo seed SFE-CO<sub>2</sub> extractions at 400 bar, 80 °C and 3.68 g•min<sup>-1</sup> [117] and between 39 % and 73 % for particle sizes between 1.0 and 0.5 mm for extractions at 300 bar, 80 °C and 1.67 g•min<sup>-1</sup> [106].

However, solid particles smaller than 0.54 mm (28–32 mesh) have also shown a tendency to agglomerate into solid lumps, in which the bulk resistance prevailed, limiting the surface area contact between the particles and the solvent mixture of CO<sub>2</sub> and ethanol. Multilayer packings were proposed as an alternative to avoid sample compaction for small particle samples with an average particle size of 0.32 mm; the extraction yield increased from 29 % to 40 % when the number of layers increased from 1 to 10 [73].

## 5.4. CO<sub>2</sub> flow rate and extraction time

Other parameters, namely CO<sub>2</sub> flow rate and extraction time, also collaborate for the physical-chemical interactions between the sample and solvent. Studies have reported a positive correlation between the extraction efficiency of Mo seed and solvent flow rate. An enhancement of the diffusion phenomenon was observed due to lower mass transfer resistance using a flow rate of 15 g•min<sup>-1</sup>, which also resulted in an extraction yield of up to 16 %, higher than the 8 % achieved at 5 g•min<sup>-1</sup> over 250 min of extraction, 300 bar, and 80 °C [106]. Also, flow rates ranging from 1.84 to 9.20 g•min<sup>-1</sup> have shown influence upon the extraction efficiency, either correlated to the temperature (40–120 °C) or pressure (200–600 bar). Yields of 5 % and 40 % were found for the respective CO<sub>2</sub> rates while carrying the experiments out for 30–150 min [117].

However, increasing the solvent flow rate above the optimal point - which may vary according to the extraction parameters and sample -

reduces the contact time between the supercritical fluid and the sample's surface. During the phenomenon, CO<sub>2</sub> surrounds the particle surface and cannot diffuse into the solid, restricting the extraction of the compounds and promoting particle compaction. The behaviour resembles a bypass-like process, and the system deviates from its equilibrium state [121].

Fig. 4b shows the proposed phenomenon. When extraction occurs, CO<sub>2</sub> in the absence of extract involves the particle surface by convective mechanism and then penetrates the sample through CO<sub>2</sub> diffusion. If the bypass-like process takes place, no diffusion mechanism occurs in the particle due to an external film resistance [117]; therefore, only the compounds from the outer surface layer might be extracted.

To avoid the bypass-like process, a longer extraction time can be applied. This strategy has favoured the extraction yield of Mo leaves compounds [17]. In turn, to also reduce the extraction time, small Mo seed particles have been used in response to a higher surface area and lower diffusivity resistance [45,106]. However, the mass transfer shows a limit, evidenced by the solvent saturation and the maximum accumulative extraction yield [116].

Building on results previously obtained by Kessler et al. [19], the effectiveness of CO<sub>2</sub> in extracting the main compounds from Mo leaf and seed has been calculated and is presented in Table 4. The estimated CO<sub>2</sub> consumption for Mo leaf and seed, respectively, was 0.36 and 0.40, with an equal flow rate of 4.0 mL·min<sup>-1</sup>, using pressures of 195 bar and 273 bar or CO<sub>2</sub> densities of 747 kg·m<sup>-3</sup> and 830 kg·m<sup>-3</sup> [19]. These experiments revealed that only 5.0 kg of CO<sub>2</sub> per gram of compound was required for extracting oleic acid from seeds, while the extraction of α-tocopherol, linolenic acid, and nonacosane from Mo leaf consumed 18.1 kg·g<sup>-1</sup>, 15.9 kg·g<sup>-1</sup>, and 109.8 kg·g<sup>-1</sup>, respectively [17].

Previous studies indicate the applied CO<sub>2</sub> flow rate and extraction time condition, as well as their respective working range of pressure and temperature. Mo leaf extractions worked at 1.87 g·min<sup>-1</sup>, for 60–120 min, at 300–500 bar, and 40–80 °C [17], 1.37 g·min<sup>-1</sup>, for 25–130 min, at 150–250 bar, and 35–80 °C [18], and 3.97 g·min<sup>-1</sup>, for 120 min, at 117–273 bar, and 41–69 °C; while Mo seed extractions were performed using 0.66 g·min<sup>-1</sup>, for 40 min, at 250–350 bar, and 40–60 °C [100], and kg7.5 g·min<sup>-1</sup>, for 420 min, at 150–300 bar, and 35–60 °C [105], for example.

## 6. Statistical alternatives to ensure the efficiency of extraction methodologies

Current expectations face a two-fold issue: high productivity of high-quality products. To meet both expectations, studies have been using statistical methodologies as a strategy to ensure production efficiency, as well as to assure the reproducibility of the data and products.

By conventional and green extraction methodologies, experimental and mathematical tools have been considered to reach a target compound composition from natural sources [18,116,117,122]. Therefore, this section is dedicated to showing the use of statistical methods and the contribution of the fitted parameters in the isolation of compounds from the Mo tree.

Among the main statistical alternatives, the response surface methodology (RSM) associated with the design of experiments (DOE) has been extensively used [19,42,53,105,117]. Both analyses are a set of mathematical and statistical procedures to refine, optimise and develop

an engineering process [99]. In SFE-CO<sub>2</sub> studies, several efforts have been performed to improve both compound selectivity and extraction yield by optimising pressure, temperature, particle size, co-solvent concentration, CO<sub>2</sub> solvent flow, and extraction time [105,123].

A full-factorial DOE considering the pressure and temperature effects on the extraction yield was carried out using Mo seed samples [42]. The fitted model ( $R^2 \geq 0.94$ ) predicted the best condition for obtaining non-polar compounds and indicated the pressure as a significant parameter ( $p=0.001$ ). DOE and RSM methodologies were combined in Mo seed SFE-CO<sub>2</sub> extractions using five distinct levels of temperature, pressure, extraction time, and flow rate [117]. Pressure/temperature and pressure/flow rate interactions significantly and positively influenced the extraction yield. However, pressure/extraction time, temperature/flow rate and temperature/extraction time negatively affected the extract recovery ( $R^2=0.97$ ,  $p=0.0001$ ).

The independent variables of temperature, pressure, and particle size were considered in SFE-CO<sub>2</sub> experiments of Mo seed analysed by RSM [105]. The quadratic model - second-order terms - performed well for the evaluated parameters ( $p=0.0001$ ), and a significant influence on the extraction yield ( $\alpha=0.05$ ) was observed regarding the pressure and temperature factors. According to this study, the pressure statistically affected the results due to the CO<sub>2</sub> solvent power effect. Pressure has also been reported as the main influence over the SFE-CO<sub>2</sub> extraction of Mo seeds, leaves, and roots by applying DOE and RSM combined [19]. These findings allowed the optimisation towards target compounds from the sample materials using a quadratic model to fit the experimental data ( $R^2 \geq 0.95$ ). Moreover, the flavonoid composition of Mo leaves could be evaluated regarding pressure and temperature by using RSM [53].

The Box complex optimisation and Sovova model were used to study the influence of SFE-CO<sub>2</sub> factors [93]. The authors identified a dominant response of the diffusion mechanism into Mo seed, mainly due to the pressure, solvent flow rate, and co-solvent effects.

The ratio of Mo seed:solvent and extraction time were also evaluated in MAC extractions [99]. The D-optimal design was adopted to analyse the results. This methodology can minimise the variance of the regression coefficient and, combined with the RSM, allows the use of qualitative and quantitative variables. In this regard, no significant difference ( $\alpha=0.05$ ) was observed in the quadratic regression model ( $R^2=0.99$ ,  $p<0.0001$ ) considering all interaction factors. The model was interpreted as adequate due to the agreement between the predictive responses and the observed yield.

SOX extractions were also accomplished using a central composite rotatable design (CCRD) and RSM. The influence of particle size, extraction temperature, and residence time were considered. The optimised parameters indicated 0.62 mm, 56.5 °C, and 420 min, and the regression coefficients ranged from 0.77 to 0.93 according to the extraction solvent [98]. Statistical tools have been explored to enhance the obtention of phytochemicals from the Mo tree [19,42,105,117]. The optimisation of extraction yield and target composition is a functional approach to insert new cosmetic ingredients on the market; α-tocopherol and oleic acid were found to be suitable bioactives to be extracted by SFE-CO<sub>2</sub> from Mo leaves and seeds, respectively, as antioxidant agents after DOE/RSM analyses [19]. Cosmeceutical products containing phenolic-rich Mo leaf hydroethanolic extract (MAC) were statistically analysed towards the extract obtainment (yield, phenolic content, and antioxidant activity) and emulsion stability (viscosity and pH) [31]. A

**Table 4**  
Estimated kg of CO<sub>2</sub> circulated per gram of compound extracted from Mo leaf and seed.

Plant part	Compound	kg CO <sub>2</sub> *	g extract	mg compound·g extract <sup>-1</sup> *	g compound	kg CO <sub>2</sub> ·g compound <sup>-1</sup>
Leaf	α-tocopherol	0.36	0.13	148	$1.98 \times 10^{-2}$	18.1
	Linolenic acid		0.13	168	$2.25 \times 10^{-2}$	15.9
	Nonacosane		0.13	24	$3.26 \times 10^{-3}$	109.8
Seed	Oleic acid	0.40	0.34	238	$8.04 \times 10^{-2}$	5.0

\* Data adapted from Kessler et al. [19].

body wash formulation has increased its antioxidant activity by introducing Mo leaf extract obtained by UAE [70]. The extracts and respective activities have previously been optimised using ANOVA and Bonferroni post-test (not discussed in this review).

The evidence obtained by statistical analyses and their improvement in process efficiency surpasses the scientific database. The mathematical study can also improve the confidence to scale up an industry process by uncovering patterns and trends to achieve high productivity of high-quality products. These approaches are described for an SFE-CO<sub>2</sub> extractor by applying the Sovova model [124] and in a techno-economic optimisation of the Mo seed sterol fraction by integrating RSM with the cost of manufacturing [123].

## 7. Conclusions

This work has shown the main findings regarding Mo's phytochemical properties and their use in cosmetic products. Patents and scientific publications have summed, over the last 25 years, 72 and 123 documents, respectively, by matching the keywords "*Moringa oleifera*" and "cosmetic". The data claims the recognition of anti-ageing and photoprotective effects of antioxidant and anti-inflammatory compounds, such as flavonoids, phenolics, fatty acids, and vitamins. These properties have been explored in sunscreen and various skin care formulations to both increase the oxidative stability of Mo-based products and reduce the skin's oxidative stress. Mo extracts have been found to be more often linked to wound healing, drug, and green synthesis keywords due to their antibacterial, antifungal, and analgesic activities, which have been highly noticed in the last three years.

Beyond the feasibility of Mo bioactives, this study has shown an understanding of the main effects on extraction yield and extract selectivity, especially regarding the SFE-CO<sub>2</sub>. The reviewed studies on SFE-CO<sub>2</sub> extraction of Mo seeds and leaves have repeatedly found the pressure as a significant parameter, mostly related to the extraction of non-polar and low-polar compounds (e.g. fatty acids, sterols, and tocopherols). The solvation power of the supercritical CO<sub>2</sub> towards the extraction of phenolic acids, flavonoids, and organic acid compounds can be increased by using ethanol or water as co-solvents. Meanwhile, the temperature may increase the solubility of the extractable compounds in CO<sub>2</sub> but also degrade the thermolabile ones by reducing their bioactive potential. Lastly and despite the limited research, particle size and extraction time have emerged as factors that influence CO<sub>2</sub> diffusivity.

The increased effort of the scientific community has enhanced the reliability of Mo extracts as a safe and active ingredient. However, the scarcity of studies on its use in cosmetic formulations shows that Mo's natural value and ready availability remain underexplored even after centuries of empiric acknowledgement and decades of scientific research. This review has identified further opportunities for study: optimising cosmetic formulations using Mo extract as an ingredient, with the goal of developing marketable products, and employing stabilisation methods such as microencapsulation to preserve the bioactive properties of Mo.

## CRedit authorship contribution statement

**Júlia C. Kessler:** Writing – original draft, Methodology, Investigation, Conceptualization. **Isabel M. Martins:** Writing – review & editing, Data curation. **Yaidelin A. Manrique:** Validation, Formal analysis. **Alfrio E. Rodrigues:** Writing – review & editing, Supervision. **Maria Filomena Barreiro:** Writing – review & editing, Supervision, Funding acquisition. **Madalena M. Dias:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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