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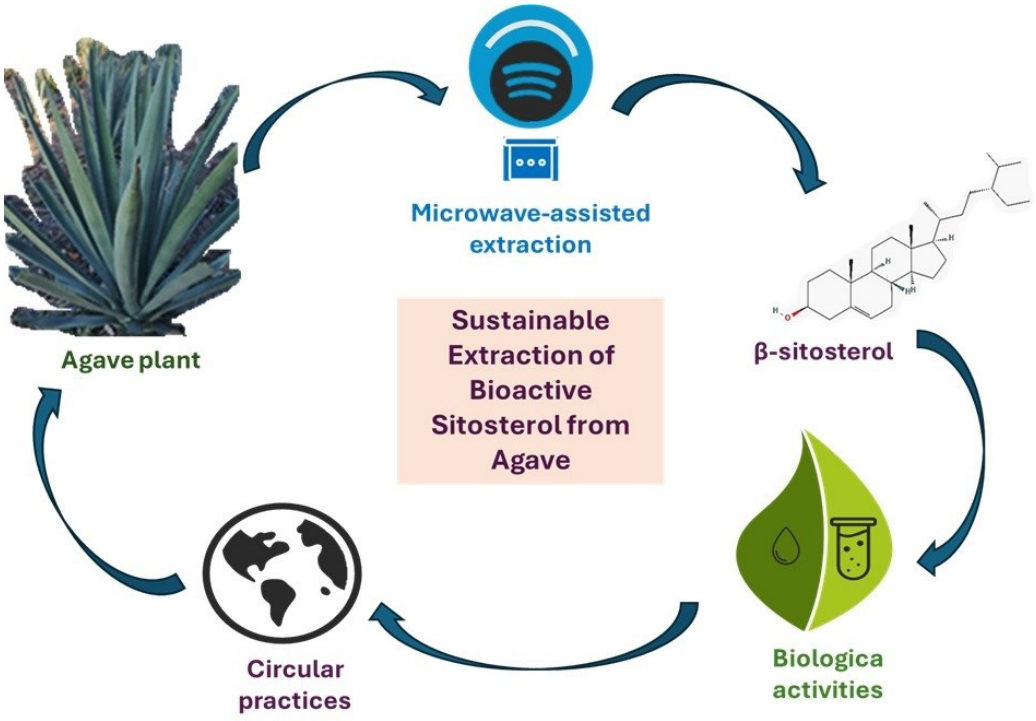
GRAPHICAL ABSTRACT



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Agave species are increasingly recognized as promising sources of bioactive phytochemicals with therapeutic potential. Among these, β-sitosterol (BSS) and its glucoside (BSSG) have gained attention for their wound-healing, anti-inflammatory, antioxidant, and immunomodulatory properties. *In vitro*, these compounds enhance fibroblast viability and regulate cytokine production. *In vivo*, extracts from *Agave angustifolia* bagasse (BagEE), obtained through microwave-assisted extraction (MAE), significantly accelerate wound closure and re-epithelialization. MAE, particularly when combined with alkaline catalysts, yields higher concentrations of BSS and BSSG compared to conventional methods. However, despite its environmental and efficiency advantages, supercritical fluid extraction remains underutilized for isolating phytosterols from *Agave*. This review highlights interspecies variation in bioactive profiles, the critical impact of extraction methodology on compound yield and activity, and the potential for valorizing agro-industrial residues. These findings emphasize the value of *Agave*-derived sterols in the development of sustainable, plant-based therapeutics. Further research is needed to standardize extraction protocols, achieve comprehensive characterization of active metabolites, and evaluate their clinical efficacy—advancing innovation in bioproduct development aligned with circular economy principles.

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Keywords: Circular economy; Agave bagasse; Agave leaves; Waste; CAM

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INTRODUCTION

Agave plants, commonly known as "maguey," have played a fundamental role in Mexican culture and history since ancient times. These species hold substantial ecological, socio-cultural, and economic significance, with Mexico recognized as the center of origin and diversification of the *Agave* genus (Colunga-GarcíaMarín *et al*. 2007). The genus *Agave* comprises approximately 210 species, with Mexico harboring 129 endemic and a total of 159 species within its borders (Colunga-GarcíaMarín *et al*. 2007; García-Mendoza *et al*. 2019). Some *Agave* species have been introduced to other continents, including Africa and Asia (Lim 2012), expanding their global importance. Figure 1 depicts a young *Agave angustifolia* Haw plant representing the early stages of its development.

Fig. 1. Photograph of a young *A. angustifolia* plant, showcasing its early growth stages

Their remarkable genetic diversity has enabled diverse applications, from food and syrup production to fiber extraction, fermented beverages, and bioactive compound generation (Sidana *et al*. 2016).

Scientific interest in *Agave* has grown considerably in recent years, leading to an increasing number of studies exploring its biochemical composition, ecological roles, and potential applications. Table 1 summarizes key areas of recent *Agave* research, highlighting advances in bioactive compound characterization, agroforestry significance, domestication processes, and biotechnological applications. Recent studies have also focused on sustainable management strategies and the utilization of *Agave* by-products, which aligns with contemporary efforts toward green and circular economies (Alducin-Martínez *et al*. 2022; Álvarez-Chávez *et al*. 2021).

Table 1. Areas of *Agave* Research

Research	Reference
Ways in which *Agave*s benefit human health and nutrition	(Santiago-Martínez *et al*. 2023)
Sustainable Management of *Agave*s	(Alducin-Martínez *et al*. 2022)
Agave fructans	(Espinosa-Andrews *et al*. 2021)
Socioeconomic and agroecological importance	(Blas-Yañez and Thomé-Ortiz 2021)
Agave by-product utilization	(Álvarez-Chávez *et al*. 2021)
Management and domestication	(Álvarez-Ríos *et al.* 2020)
Content of bioactive compounds	(López-Salazar *et al*. 2019)
Significance in agroforestry systems in Mexico	(Torres-García *et al*. 2019)
Fermented beverages with deep roots in Mexican tradition	(Ramírez-Guzmán *et al*. 2019)
Source for Bioenergy	(Ruiz *et al*. 2016)
Agave biotechnology	(Nava-Cruz *et al*. 2015)
Genetics of Mexican *Agaves*	(Suárez-González *et al*. 2014)
Plant cell cultures	(Kartosentono *et al*. 2002)

One of the most notable characteristics of *Agave* plants is their use of crassulacean acid metabolism (CAM), a specialized photosynthetic pathway that enhances water-use efficiency. This allows them to thrive in arid environments, making them highly relevant in the context of climate change (Stewart 2015). Their ability to accumulate soluble carbohydrates and secondary metabolites in large vacuoles further enhances their resilience and economic potential. Recent studies have highlighted the significance of CAM's role in maintaining productivity under extreme environmental conditions, positioning *Agave* species as promising candidates for future agricultural and industrial development (Davis and Ortiz-Cano 2023).

Due to increasing demand for sustainable bioresources, *Agave* has attracted attention as a source of bioactive compounds, including phytosterols such as BSS and its glycosylated derivative. Both have demonstrated pharmacological potential, including anti-inflammatory, antimicrobial, cholesterol-lowering, and anticancer effects (López-Salazar *et al*. 2022b; Santiago-Martínez *et al*. 2023). However, despite their promise, research on their sustainable extraction from *Agave* and biotechnological utilization remains limited.

Recent methodological developments have addressed this gap. In 2019, López-Salazar *et al*. identified and quantified BSSG in ethanolic extracts from *A. angustifolia* stems using microwave-assisted extraction (MAE). This technique yielded up to 125 mg/g dry weight (DW) in just 5 seconds—compared to only 26.7 mg/g DW after 48 hours of conventional maceration—while preserving compound integrity (López-Salazar *et al.* 2019).

Further progress was reported in a 2022 study, where the same group extracted both BSS and BSSG from *A. angustifolia* bagasse using MAE, obtaining yields of 103.6 mg/g for BSS and 61.6 mg/g for BSSG (López-Salazar *et al.* 2022a). This work highlighted the potential of *Agave* agro-industrial residues as abundant sources of bioactive compounds, aligning with circular economy goals and phytochemical valorization strategies.

Beyond extraction, recent studies have also confirmed the pharmacological relevance of these compounds. In a 2025 study, a microwave-assisted ethanolic extract from BagEE, which included BSS and BSSG among its main components, significantly enhanced wound healing in a murine excision model. Treated wounds showed 99.4% closure by day 13, compared to 92.8% by day 22 in controls. Histological analysis confirmed complete re-epithelialization and improved collagen structure, supporting the regenerative properties of the extract (López-Salazar *et al*. 2025).

Taken together, these findings highlight impactful recent advances in *Agave*-derived bioactives, especially regarding sustainable extraction and biomedical potential. They also underscore the need to further explore these compounds within the frameworks of green chemistry and circular bioeconomy.

The objective of this review is to provide a comprehensive overview of the sustainable utilization of BSS and BSSG derived from *Agave* species. It explores recent progress in green extraction technologies, evaluates the pharmacological potential of these compounds, and identifies opportunities for innovation in sustainable agriculture, biotechnology, and health sciences. This work emphasizes the importance of *Agave* in supporting circular economic practices and addressing current environmental and socio-economic challenges.

Unlike previous reviews, this manuscript uniquely integrates the latest research on eco-friendly extraction techniques and the pharmacological activity of BSS and BSSG

specifically from *Agave* species. It also critically assesses existing knowledge gaps, offering a novel framework for future studies and potential therapeutic applications.

To gather and analyze the scientific literature discussed in this chapter, a comprehensive search was conducted in major academic databases, including PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar. Keywords such as *Agave*, *Agave* bagasse β-sitosterol, β-sitosterol glucoside, biological activity , extraction methods, microwave-assisted extraction, and phytochemical composition were used. The selection focused on peer-reviewed studies related to the biological activities and bioactive compound content of *Agave* species, with emphasis on recent findings that support sustainable bioproduct development.

To ensure the scientific rigor of this review, the selection of studies was based on specific inclusion criteria. Only peer-reviewed original research articles published between 2010 and 2025 were considered. The focus was placed on studies reporting the presence, extraction, or biological activity of β-sitosterol and/or β-sitosterol glucoside specifically derived from *Agave* species. Preference was given to studies employing sustainable or green extraction techniques, such as microwave-assisted extraction, and those that evaluated pharmacological effects *in vitro* or *in vivo*. Articles were also selected based on the identification of compounds using analytical methods such as HPLC or mass spectrometry. Reviews, articles lacking phytochemical characterization, or those unrelated to *Agave* were excluded from the analysis. This approach allowed for a focused and critical synthesis of relevant, high-quality data concerning the bioactivity and therapeutic potential of *Agave*-derived phytosterols.

Agave Sustainability Through Circular Practices

Agave plants have been utilized for a wide array of purposes, including as a source of food, medicines, alcoholic drinks, vinegar, fibers such as ixtle, fertilizers, building materials, and decorative items. In the culinary realm, *Agave*s represent a significant bioresource due to their ability to produce various distilled and fermented beverages, as well as dietary fibers with prebiotic properties (Santiago-Martínez *et al*. 2023).

In some *Agave* species, an inflorescence termed the “quiote” grows from the plant’s center and can produce flowers. Since these plants flower only once in their lifetime, they are considered monocarpic. They reproduce either sexually by seeds or asexually through their vegetative stem (Pérez-Zavala *et al*. 2020).

Mexico is widely recognized as a leading producer of *Agave*-based beverages such as tequila, mezcal, bacanora, and pulque, leveraging the country’s extensive agave biodiversity. In response to growing global demand, production volumes have increased substantially, resulting in a significant accumulation of *Agave* waste rich in lignocellulosic material. This biomass is often used to produce biofuels and bioplastics (Estrada-Maya and Weber 2022).

Agave waste also serves as a cost-effective raw material for generating food additives, rheological modifiers, and high-value chemical compounds, offering substantial potential for developing functional foods with health benefits. The production of affordable food ingredients or products with targeted functionalities from lignocellulosic biomass aligns with the principles of a circular economy, which seeks to minimize raw material waste (Sabater *et al.* 2021; Sovljanski *et al*. 2023).

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According to the Tequila Regulatory Council (2022), tequila production increased by 345 million liters over the past 21 years, reflecting a 190% growth since 2000. From 2000 to 2010, the average annual growth rate was 4.69%, rising to 7.54% between 2011 and 2021. The period from 2016 to 2021 marked the highest average annual growth (15.6%). By the end of 2021, tequila exports reached a record value of $3.3 billion. Export growth averaged 15.0% annually between 2011 and 2021, and 19.2% during 2016–2021. From 2000 to 2021, the total value of exports increased by 656%, and export volume rose by 244% (240.6 million liters), with the highest average annual increase (11.0%) reported from 2016 to 2021 (Díaz, 2023). These figures not only emphasize the economic relevance of the tequila industry but also underscore the urgent need for sustainable *Agave* waste management through circular economy approaches.

As tequila continues to gain popularity in more than 50 countries, global demand is expected to rise. Consequently, there is a growing interest in fully utilizing the *Agave* plant to reduce waste and support circular economy initiatives (Alcazar-Valle *et al*. 2019).

These strategies enable the transformation of *Agave* residues, particularly bagasse and leaves, into food ingredients, biofuels, biofertilizers, and other sustainable materials (Sabater *et al.* 2021; Sovljanski *et al*. 2023). For instance, Honorato-Salazar *et al.* (2021) demonstrated the potential of *Agave* and nopal as sustainable raw materials for bioenergy and co-products, highlighting the efficient use of lignocellulosic biomass within circular economy frameworks. More recently, Warren-Vega *et al*. (2025) reported the use of *Agave* bagasse as a biotemplate for producing sustainable materials, recovering valuable metabolites, and generating energy, reinforcing the feasibility of a circular biorefinery model.

Agave plants consist of two primary components: the large, spiny leaves—ranging from broad to narrow and arranged in a rosette formation and the stem, commonly referred to as the "piña" or "pineapple" (Fig. 2). The stem is traditionally cooked to extract juices used in the production of various beverages, such as mezcal and tequila, the latter being the most widely consumed Mexican alcoholic beverage worldwide (Ramírez-Guzmán *et al*. 2019).

Fig. 2. Agave stem or "pineapple"

Residual *Agave* Materials: Leaves and Bagasse

During the production of distilled alcoholic beverages, *Agave* plants undergo an annual trimming process to promote the growth of their central core, or pineapple. At the time of harvest—typically when the *Agave*s are between 7 and 8 years old, all the leaves are removed in a process known as Jima. Following this step, the stems, which constitute approximately 40% of the plant's wet weight, are cooked, shredded, and ground to extract sugars necessary for alcoholic fermentation using various technological methods. It is estimated that 6 to 8 kg of agave are required to produce one liter of tequila (Estrada-Maya and Weber, 2022).

Approximately half of the total weight of an *Agave* plant consists of its leaves. After the "Jima" process, these leaves are usually discarded and left on the soil surface, as illustrated in Fig. 3.

Fig. 3. The "Jima" Process

This practice poses an environmental risk, as the decomposing leaves can become breeding grounds for pathogenic microbes and parasites (Pérez-Zavala *et al.* 2020).

Agave leaves are rich in lignocellulosic material and tough fibers, making them a significant by-product of the distillation industry. Due to their high fiber content, there is growing interest in using them to develop innovative, compostable biocomposites. These fibers composed primarily of cellulose, hemicellulose, lignin, pectins, waxes, and water-soluble compounds are especially attractive for producing composite materials because of their favorable mechanical and physical properties (Márquez-Rangel *et al*. 2023).

Traditionally, *Agave* leaves have also been used as food, particularly in Mexico. For example, leaves from *Agave salmiana* are used to line underground ovens in the preparation of traditional dishes such as barbacoa, where goat, sheep, or beef is slow-cooked (Santiago-Martínez *et al.* 2023). Additionally, various (pp. 605-635). *Agave* leaves contain secondary metabolites with beneficial biological properties for human health (Hernández-Valle *et al.* 2014).

Agave bagasse, the fibrous residue left after cooking and crushing the stems to extract sugars, accounts for roughly 40% of the total processed *Agave* weight in its wet form. Bagasse is composed of 41 to 45% cellulose, 19 to 25% hemicellulose, 15 to 20% lignin, and 6 to 7% ash (Estrada-Maya and Weber 2022). The *Agave* bagasse holds substantial potential to produce various items. These include filters, absorbents,

geotextiles, fiberboard, packaging materials, molded products, production of biofuels and secondary metabolites with medicinal properties (Moreno-Anguiano *et al*. 2022).

Among the valuable compounds derived from *Agave* is a class of secondary metabolites known as fructans, which have gained attention due to their broad spectrum of applications and health-promoting properties. Primarily extracted from the stem, *Agave* fructans are known for their prebiotic activity and potential applications in functional food and pharmaceutical products. However, because fructans extraction focuses almost exclusively on the stem, large amounts of leaves and bagasse remain as underutilized residues. The following section explores the characteristics and importance of agave-derived fructans in greater detail

Important *Agave*-Derived Compounds: Fructans

Fructans, like the sugars used in the production of fermented beverages such as mezcal and tequila, are extracted from the stem of the *Agave* plant, which functions as a natural carbohydrate reservoir. These polysaccharides consist of fructose polymers with varying degrees of polymerization (DP) and structural complexity (Espinosa-Andrews *et al*. 2021).

Fructans located in the stem and basal leaves of *Agave*s serve as an essential energy reserve for plant growth, constituting between 60% and 85% of the soluble carbohydrates in these species. Found in species such as *Agave tequilana, A. angustifolia, A. patatarum, A. salmiana,* and *A. fourcroydes*, these fructans include complex mixtures of β(2→1) and β(2→6) linkages. Preclinical studies with agavins from *A. tequilana* have shown beneficial effects on glucose and lipid metabolism in male mice, along with body weight reduction in hypercholesterolemic rats. Low-DP fructans (<10) from *A. tequilana* decreased weight gain by 30%, reduced fat mass by 51%, hepatic steatosis by 40%, and hyperglycemia by 25% in obese mice. Furthermore, low-DP fructans from *A. angustifolia* and *A. patatarum* increased the secretion of appetite-regulating peptides, contributing to obesity management and the treatment of metabolic disorders (Santiago-Martínez *et al*. 2023).

Inulin, another major carbohydrate in the *Agave*, is a type of fructans consisting of β(1→2) fructosyl-fructose linkages. Clinical studies have shown that daily intake of 10 grams of inulin over eight weeks significantly reduces blood glucose, insulin, C-reactive protein, TNF-α, and lipopolysaccharide (LPS) levels in individuals with type 2 diabetes. LPS, a component of Gram-negative bacterial membranes, is implicated in metabolic decline associated with obesity and diabetes. Inulin-type fructans are non-digestible, fermentable, soluble, and non-viscous fibers. They modify gut microbiota by promoting the growth of *Bifidobacteria* and *Bacteroidetes*, while reducing *Firmicutes*, thereby alleviating dysbiosis commonly associated with metabolic diseases (Santiago-Martínez *et al*. 2023).

Because the *Agave* genus utilizes CAM, these species are capable of photosynthetically generating fructans that function as osmoprotectants during periods of drought. Thus, inulin and other fructans are stored as reserve carbohydrates. The most common method for recovering inulin involves conventional hot-water extraction at 80 °C with agitation. Various analytical techniques are subsequently used to characterize the resulting inulin-rich powder (Apolinario *et al*. 2017).

Recently, *Agave fructans* have been incorporated into functional foods due to their beneficial technological properties and health effects. These include the stimulation of beneficial gut bacteria, modulation of serum glucose levels, reduction of obesity-

related disorders, improved calcium absorption, and chemoprotective, immunomodulatory, and antioxidant benefits (Espinosa-Andrews *et al*. 2021).

Fructans extraction also relies on the stem, generating substantial residues in the form of leaves and bagasse. However, despite growing industrial interest in *Agave fructans*, the volume and management of residual biomass from *fructans* extraction remain poorly documented highlighting an important gap for future sustainability evaluations.

Bioactive Compounds Extracted from *Agave* Plants

Diversity of bioactive compounds in Agave

Agave plants contain a variety of secondary metabolites, including flavonoids (Morreeuw *et al.* 2021), homoisoflavonoids (Morales-Serna *et al*. 2010), phenolic acids (Almaraz-Abarca *et al.* 2013), tannins (Morán-Velázquez *et al*. 2020), volatile coumarins (Soto-Castro *et al.* 2021), long-chain alkanes, fatty acids and alcohols (Rizwan *et al.* 2012). Additionally, they contain steroidal sapogenins and saponins, as well as sterols (García-Morales *et al.* 2022; López-Salazar *et al*. 2022).

Steroidal sapogenins and saponinis in Agave

Steroidal sapogenins and saponins are the most widely studied compounds in this genus. *Agave* is a major source of steroidal sapogenins, mainly of the spirostanol type (1e27), with Agavegenin D being the only cholestane-type sapogenin identified in the genus to date. Skeletons of furostanol and furospirostanol have not been found in *Agave*. Spirostanols, derived biogenetically from cholestane, have a 16,22; 22,26-bisepoxycholestane structure. The spirostanol skeleton comprises a tetrahydrofuran ring (E) and a tetrahydropyran ring (F) joined at C-22 in a spiran configuration. These compounds are extracted from various parts of *Agave* plants, including leaves, flowers, leaf juice, rhizomes, and callus cultures. Spirostan sapogenins from *Agave* differ in their hydroxyl group configurations and numbers on the parent nucleus, the presence or absence of a carbonyl group at C-12, the saturation state of rings B or C, and the configurations of hydrogen atoms at C-5 and C-25, influenced by biogenetic factors (Sidana *et al*. 2016).

Structure, classification and ciological activity of saponins

Saponins consist of a hydrophobic aglycone (sapogenin) linked to a hydrophilic sugar (glycone). *Agave* saponins contain β-D-glucopyranosyl, β-D-galactopyranosyl, β-D-xylopyranosyl, and α-L-rhamnopyranosyl units. They are classified as spirostanol or furostanol glycosides according to the sapogenin nucleus and further subdivided by the number of attached sugars (mono- to hexaglycosides). Monodesmosidic saponins bearing a single sugar at C-3 predominate among *Agave* spirostanol glycosides, whereas bidesmosidic saponins are rare among spirostanols but common in furostanol counterparts. Extraction typically relies on conventional Soxhlet or maceration methods. Notably, *Agave* saponins and sapogenins are recognized for their antimicrobial and anticancer properties (Sidana *et al*. 2016).

Following the background discussion on *Agave* sustainability and the various bioactive compounds derived from this plant, attention now turns to BSS. This section will explore its extraction methods and biological activities, highlighting its significance in the broader context of *Agave* utilization, including its residues.

β-Sitosterol

β-Sitosterol (BSS), depicted in Fig. 4, is a phytosterol classified as a secondary metabolite (SM). While SMs are not directly involved in essential functions such as growth, development, or reproduction, they play crucial ecological roles, including plant defense against herbivores and pathogens, and the attraction of pollinators (Ferrer *et al*. 2017). Phytosterols also exhibit a broad spectrum of biological activities, which have attracted considerable interest due to their relevance to human health and well-being (Chanioti *et al.* 2021).

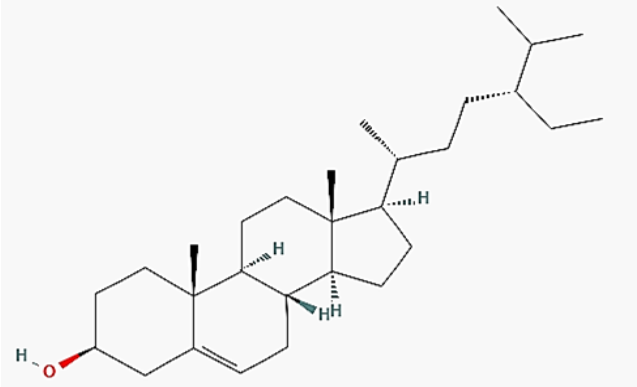

Fig. 4. The chemical structure of β-sitosterol (BSS) (Bin *et al*. 2016) obtained from PubChem PubChem CID 222284

In plants, they are key structural components of eukaryotic cell membranes, modulating membrane fluidity and permeability. Moreover, they serve as precursors in the biosynthesis of brassinosteroids, plant hormones essential for morphogenesis, development, and responses to biotic and abiotic stresses (Ferrer *et al.* 2017).

Phytosterols are found both as free sterols (FS) and in conjugated forms such as steryl esters (SEs), steryl glycosides (SGs), and acyl steryl glycosides (ASGs). In SEs, the hydroxyl group at the C3 position is esterified with a fatty acid. SGs are defined by the presence of a sugar molecule attached to the C3 hydroxyl group of the sterol structure through a β-glycosidic bond. ASGs are derivatives of SGs where the hydroxyl group at the C6 position of the sugar moiety is esterified with a fatty acid (Moreau *et al.* 2002).

FSs are synthesized in the endoplasmic reticulum (ER) and delivered to the plasma membrane (PM) *via* the secretory pathway. Glycosylation and subsequent acylation, yielding SGs and ASGs, respectively, occur at the PM (Beck *et al*. 2007). Their insertion into lipid bilayers regulates lipid chain ordering and phase behavior, promoting the formation of an intermediate liquid-ordered phase characterized by high molecular mobility and structural organization. This ensures optimal membrane fluidity, permeability, and mechanical properties (Beck *et al.* 2007).

Free sterols account for approximately 70 to 90% of total sterols in the PM of various plant species and tissues, including rhizomes, leaves, and fruits (Bin *et al.* 2016). The primary phytosterols in plants are BSS, stigmasterol, and campesterol. BSS and stigmasterol are involved in membrane structure and function, while campesterol serves as a brassinosteroid precursor (Ferrer *et al*. 2017).

BSS is a white, waxy solid with a melting point between 139 and 142 °C and a molecular formula of $C_{29}H_{50}O$. Structurally, it is like cholesterol, differing by a double bond between C5 and C6. It is thermally unstable and susceptible to oxidation (Bin *et al.* 2016).

Biosynthetically, BSS is mainly synthesized through the mevalonate pathway, although the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway can also be involved under specific environmental conditions. 13C-labeled precursor studies have shown that isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) condense to form farnesyl diphosphate (FPP), which dimerizes to form squalene. Squalene then cyclizes into cycloartenol, which is further converted into BSS through methylation, hydride shifts, and reduction reactions (Kongduang *et al.* 2008).

Since humans cannot synthesize phytosterols, dietary intake is essential. For this reason, BSS is found in both natural foods and fortified products such as margarine and salad dressings. The esterified form is preferred due to its higher lipid solubility. BSS accounts for approximately 65% of the phytosterols used in functional foods, followed by campesterol (30%) and stigmasterol (3%) (Ogbe *et al*. 2015).

BSS has been extensively studied for its biological activities, including antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties (Ogbe *et al.* 2015). It has been approved by the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) for use in cholesterol-lowering formulations, with effective doses ranging between 1.5 and 2.4 g per day (Saeidnia *et al*. 2014).

In prostate cancer studies, BSS reduced cell proliferation by 24%, increased apoptosis fourfold, and raised cellular ceramide levels in LNCaP cells without affecting prostate-specific antigen levels. It also inhibited 22Rv1 and DU145 cell lines, potentially through the activation of protein phosphatase 2A and changes in membrane fluidity (Jourdain *et al.* 2006).

In pancreatic cancer models, BSS induced G0/G1 arrest, promoted apoptosis, inhibited NF-κB, and downregulated epithelial–mesenchymal transition (EMT) markers. These effects were associated with modulation of the AKT/GSK-3 signaling pathway. Furthermore, co-treatment with gemcitabine demonstrated synergistic effects both *in vitro* and *in vivo* (Cao *et al*. 2019).

In colon cancer, BSS significantly inhibited the proliferation of HT-29 cells by altering membrane cholesterol and sphingomyelin content, suggesting disruption of lipid-mediated signaling pathways (Awad *et al.* 1996). In breast cancer cell lines MDA-MB-231 and MCF-7, BSS induced apoptosis *via* activation of caspases (Chai *et al.* 2008). In gastric cancer (AGS cells), it promoted apoptosis through modulation of AMPK, PTEN, and Hsp90, and was also associated with autophagy via PI3K/AKT/mTOR signaling (Sun *et al*. 2019).

The anti-inflammatory activity of BSS has been confirmed in various animal models. In the rat paw edema model, BSS reduced inflammation by 50 to 70%. In carrageenan-induced pleurisy, it decreased exudate volume and neutrophil infiltration, and in the mouse ear edema model, it inhibited myeloperoxidase activity by 75% (Paniagua-Pérez *et al.* 2017). BSS isolated from *Justicia gendarussa* and *Nyctanthes arbortristis* significantly inhibited the release of inflammatory mediators and cytokines such as TNF-α, IL-1β, IL-6, and reactive oxygen species (ROS). It also suppressed caspase-1 activation and inhibited NLRP3 inflammasome assembly. Nanoparticle-based delivery systems have enhanced their bioavailability and pharmacological effects (Phatangare *et al*. 2017; Nirmal *et al*. 2012).

Regarding antioxidant properties, BSS isolated from *Arisaema utile* demonstrated radical scavenging activity in the DPPH assay. At a concentration of 100 µg/mL, it exhibited 74.2% inhibition, which was comparable to the effect of the standard compound butylated hydroxytoluene (BHT) at the same concentration (75.3%).

Moreover, in the hydrogen peroxide scavenging assay, BSS showed 69.6% inhibition at 100 µg/mL, slightly higher than BHT (67.2%) (Kumar *et al.* 2017). These findings support the potential of BSS as a natural antioxidant agent with applications in both the pharmaceutical and food industries.

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In colon cancer, BSS significantly inhibited the proliferation of HT-29 cells by altering membrane cholesterol and sphingomyelin content, suggesting disruption of lipid-mediated signaling pathways (Awad *et al.* 1996). In breast cancer cell lines MDA-MB-231 and MCF-7, BSS induced apoptosis via activation of caspases (Chai *et al.* 2008). In gastric cancer (AGS cells), it promoted apoptosis through modulation of AMPK, PTEN, and Hsp90, and was also associated with autophagy via PI3K/AKT/mTOR signaling (Sun *et al*. 2019).

The anti-inflammatory activity of BSS has been confirmed in various animal models. In the rat paw edema model, BSS reduced inflammation by 50 to 70%. In carrageenan-induced pleurisy, it decreased exudate volume and neutrophil infiltration, and in the mouse ear edema model, it inhibited myeloperoxidase activity by 75% (Paniagua-Pérez *et al*. 2017). BSS isolated from *Justicia gendarussa* and *Nyctanthes arbortristis* significantly inhibited the release of inflammatory mediators and cytokines such as TNF-α, IL-1β, IL-6, and reactive oxygen species (ROS). It also suppressed caspase-1 activation and inhibited NLRP3 inflammasome assembly. Nanoparticle-based delivery systems have enhanced their bioavailability and pharmacological effects (Phatangare *et al*. 2017; Nirmal *et al.* 2012).

Regarding antioxidant properties, BSS isolated from *Arisaema utile* demonstrated radical scavenging activity in the DPPH assay. At a concentration of 100 µg/mL, it exhibited 74.2% inhibition, which was comparable to the effect of the standard compound butylated hydroxytoluene (BHT) at the same concentration (75.3%). Moreover, in the hydrogen peroxide scavenging assay, BSS showed 69.6% inhibition at 100 µg/mL, slightly higher than BHT (67.2%) (Kumar *et al*. 2017). These findings support the potential of BSS as a natural antioxidant agent with applications in both the pharmaceutical and food industries.

Recent research has further expanded the therapeutic potential of BSS through the development of novel derivatives. One important study explored the wound healing efficacy of BSS derivatives designed as potent Na+/K+-ATPase inhibitors. Beyond its classical role as an ion transporter, Na+/K+-ATPase also acts as a signal transducer involved in cell growth regulation through its interaction with the Src receptor complex, activating signaling pathways relevant to tissue repair (Cui *et al*. 2020).

In this context, a series of BSS-based small molecules was synthesized and tested for Na+/K+-ATPase inhibition and wound healing activity. Among the synthesized compounds, derivatives 31, 47, and 49 demonstrated improved potency, with IC_{50} values

of 3.0, 3.4, and 2.2 µM, respectively, compared to 7.6 µM for native BSS. Compound 49, bearing a methyl group on a benzyl oxime ether fragment and optimized electron-donating substituents, was particularly effective (Cui *et al*. 2020).

In vitro studies revealed that compound 49 enhanced fibroblast (L929) proliferation, migration, and soluble collagen production. *In vivo*, it significantly accelerated wound closure in a rat model. Mechanistically, this derivative activated key signaling molecules involved in tissue regeneration, including Src, Akt, and extracellular signal-regulated kinase (ERK), in a dose-dependent manner. Molecular interaction studies between compound 49 and Na+/K+-ATPase provided further insights into its high selectivity and potency. These findings underscore the potential of BSS derivatives as safe and efficient agents for promoting wound healing (Fig. 5) (Cui *et al*. 2020).

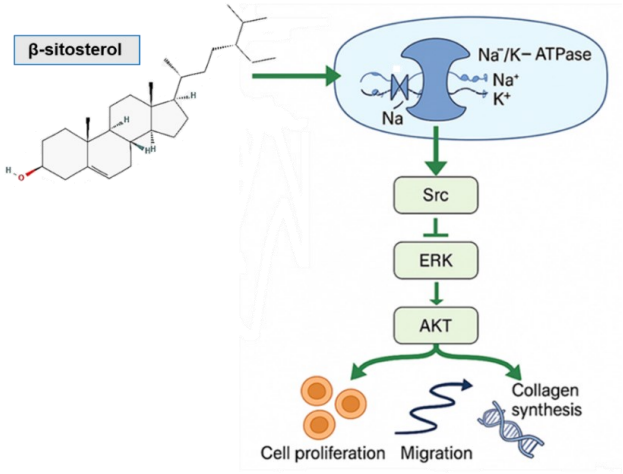

Fig. 5. Proposed mechanism of action of compound 49, a β-sitosterol derivative, showing activation of the Src/Akt/ERK signaling cascade following Na^+/K^+-ATPase inhibition (adapted from Cui *et al*. 2020)

Adipose tissue is the primary site of storage for excess energy as triglycerides and synthesizes biologically active compounds that regulate metabolic homeostasis. High dietary fat intake increases fat mass and is a major risk factor for metabolic diseases. BSS, due to its structural similarity to cholesterol, has demonstrated antidiabetic, hypolipidemic, anticancer, antiarthritic, and hepatoprotective effects. However, its impact on insulin signaling and glucose oxidation had remained unclear until recent investigations. In a study by Ponnulakshmi *et al*. (2019), BSS was administered orally (20 mg/kg/day for 30 days) to rats with type 2 diabetes induced by high-fat diet and sucrose. Results showed normalization of blood glucose, serum insulin, testosterone, lipid profile, oxidative stress markers, and antioxidant enzyme levels. Additionally, BSS increased the expression of insulin receptor (IR) and glucose transporter 4 (GLUT4) proteins in adipose tissue. In silico analyses supported these findings, suggesting that BSS may exert its antidiabetic effects by enhancing insulin signaling, positioning it as a promising therapeutic candidate in the management of type 2 diabetes.

A recent study evaluated the effect of a β-sitosterol derivative, β-sitosterol laurate (β-SLE), on serum and hepatic lipids in a hamster model. Administration of β-SLE (220 mg/5 mL oil/kg body weight) significantly reduced serum triglyceride and cholesterol levels, as well as the size of epididymal adipocytes. In addition, it protected hepatic polyunsaturated fatty acids against lipid peroxidation by activating antioxidant enzymes such as superoxide dismutase and glutathione transferase, along with reducing

malondialdehyde levels. It was concluded that the mechanism of action of β-SLE includes (i) increased fecal cholesterol excretion through reduced expression of the intestinal protein NPC1L1, and (ii) increased conversion of cholesterol to primary bile acids, induced by the activation of the enzymes cholesterol-7α-hydroxylase and sterol 27-hydroxylase. This effect was related to decreased bile acid reabsorption due to overexpression of the sodium-dependent apical bile acid transporter (ASBT) and ileal bile acid binding protein (IBABP), which together contributed to the effective reduction in serum cholesterol (Chen *et al*. 2020).

Table 2. Biological Properties of β-sitosterol (BSS) and Its Proposed Mechanisms of Action

Biological Activity	Experimental Model	Mechanism of Action/Main Findings	References
Anti-inflammatory	Rat paw edema, mouse ear edema, in vitro inflammatory cell models	Reduced edema, cytokines (TNF-α, IL-1β, IL-6), ROS; inhibited NLRP3 inflammasome and caspase-1	Paniagua-Pérez *et al*. 2017; Phatangare *et al*. 2017; Nirmal *et al*. 2012
Antioxidant	DPPH and H_2O_2 scavenging assays	High radical scavenging activity (DPPH: 74.24%; H_2O_2: 69.58%)	Kumar *et al*. 2017
Cholesterol-lowering / Hypolipidemic	Clinical and preclinical models	Decreased LDL and total cholesterol levels; increased HDL; improved lipid profiles	Ogbe *et al*. 2015; Chen *et al*. 2020
Antidiabetic	Rats with diet-induced type 2 diabetes	Normalized glucose and insulin levels; increased GLUT4 and IR expression in adipose tissue; reduced oxidative stress	Ponnulakshmi *et al*. 2019
Anticancer	Prostate, breast, colon, pancreatic, gastric cancer cell lines and animal models	Inhibited proliferation, induced apoptosis; modulated AMPK, AKT, PI3K/mTOR, NF-κB, ceramide pathways	Jourdain *et al*. 2006; Chai *et al*. 2008; Awad *et al*. 1996; Sun *et al*. 2019; Cao *et al*. 2019
Wound healing	L929 cells and rat wound model	Stimulated fibroblast proliferation, collagen production, Src/AKT/ERK pathway activation	(Cui *et al*. 2020)

The therapeutic potential of BSS has been widely investigated in various biological systems. Numerous *in vitro* and *in vivo* studies have demonstrated its efficacy in modulating key molecular pathways involved in inflammation, oxidative stress, metabolic regulation, cancer progression, and tissue repair. These findings support its application as a promising bioactive compound in pharmaceutical and nutraceutical formulations. A summary of the most relevant biological activities and underlying mechanisms of β-sitosterol reported in the literature is presented in Table 2.

Solvent Extraction Methods for Isolating Sitosterol from *Agave*

This section considers the extraction methods of phytosterols to provide a comprehensive understanding of the techniques that have been employed. The extraction and analysis of phytosterols are intricate and not yet fully standardized. As previously noted, phytosterols have significant applications in food, nutrition, pharmaceuticals, and

cosmetics. Free phytosterols obtained from plants are commonly utilized in fortified foods and dietary supplements. They are typically extracted from various plant matrices using both conventional and non-conventional methods (Uddin *et al*. 2018). To our knowledge, this is the first review to focus specifically on the extraction of phytosterols, including BSS and BSSG from *Agave* species. Therefore, an overview is first provided of general phytosterol extraction techniques to contextualize the specific methods applied to *Agave* matrices.

Different chromatographic techniques can be employed to analyze bioactive compounds, although their efficiency may vary depending on the extraction methods, including key parameters such as extraction time, temperature, and solvent polarity. The physicochemical characteristics of the target compound, as well as the complexity of the plant matrix, play critical roles in optimizing the extraction process. Therefore, qualitative and quantitative analyses of bioactive plant compounds primarily depend on the selection of appropriate and efficient extraction methodologies (Smith 2003).

Phytosterols, due to their non-polar lipid structure, are well-suited for extraction with organic solvents. Currently, both conventional and emerging green techniques are used for this purpose. Among conventional methods, Soxhlet and maceration extraction are the most established and widely referenced techniques. However, the extensive use of organic solvents in these approaches has raised concerns regarding safety, environmental sustainability, and economic cost (Chemat *et al*. 2020; Gupta *et al.* 2012). This has prompted increasing interest in greener technologies that minimize solvent usage and improve extraction selectivity.

Roiaini *et al*. (2016) investigated the impact of various extraction techniques—including Soxhlet, ultrasound, and supercritical carbon dioxide (with and without cosolvents)—on the phytosterol content of cocoa butter. Their findings revealed that the highest yields were obtained when using supercritical CO_2 with a cosolvent. Similarly, Abbas *et al*. (2010) developed an ethanol-based protocol to extract sterols from corn fiber, identifying a broad spectrum of phytosterols including α-, β-, and γ-sitosterol, sitostanol, stigmasterol, stigmastanol, campesterol, campestanol, spinasterol, and their esterified forms.

In several protocols, saponification is used to hydrolyze esterified sterols and to facilitate the analysis of both total and individual phytosterol fractions. In plant matrices, sterols are typically present in esterified forms, which require hydrolysis to release free sterols. This can be achieved either through high-pressure hydrothermal treatment (200 to 260 °C, 1.5 to 50 MPa) or by treating the material with sodium or potassium hydroxide at 90 to 120 °C under constant stirring. The latter method is preferred in many contexts because it efficiently combines hydrolysis and saponification in a single step (Rohr *et al*. 2005).

Conventional solvent-based methods include Soxhlet extraction, maceration, reflux heating, percolation, and hydrodistillation. Among these, Soxhlet extraction (a technique involving continuous hot solvent circulation), is one of the most widely used for phytosterol extraction (Uddin *et al*. 2018). Maceration, by contrast, involves soaking powdered plant material in solvent at room temperature, typically with intermittent stirring, and is considered a simpler and more accessible technique (Santiago-Martínez *et al*. 2023). Both methods have been employed for isolating sitosterols from *Agave* species.

Soxhlet extraction, while effective, requires significant time and large volumes of potentially hazardous solvents such as n-hexane, petroleum ether, ethanol, and methylene chloride. Despite these drawbacks, it remains a benchmark for comparing newer, greener

technologies. Maceration, although less solvent-intensive and lower in cost, is a time-consuming process and may be ineffective for compounds with low solubility at ambient temperatures (Vilela *et al.* 2013).

To overcome these limitations, several modern extraction techniques have emerged, including microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasonic-assisted extraction (UAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE). These methods are designed to improve yield, reduce solvent usage, and preserve the integrity of heat-sensitive bioactives (Seidel 2005). Among them, SFE, PLE, EAE, and MAE are frequently employed for sterol extraction. Of note is SFE, which offers a sustainable, solvent-free alternative with high efficiency, while MAE has been effectively used to extract sitosterols specifically from *Agave*.

MAE leverages microwave energy to heat plant material and solvents, facilitating rapid compound release. The solvent's dielectric constant is a key factor, as it determines the solvent's ability to absorb microwave energy and convert it to heat. This thermal energy enhances solute mobility and disrupts plant cell walls, improving yield. Ethanol is widely used in MAE due to its high dielectric constant, safety, and effectiveness in extracting diverse phytochemicals (Brusotti *et al.* 2014). Solvent mixtures, particularly aqueous ethanol, are often more effective because they improve microwave absorption and solvent penetration into rehydrated dry plant matrices.

Studies have shown that MAE not only reduces processing time and solvent consumption but also yields higher concentrations of BSS compared to maceration (López-Salazar *et al.* 2019). Additionally, this technique aligns with the principles of green chemistry by utilizing non-toxic solvents and energy-efficient mechanisms. While *Agave* species are most studied for their saponins and fructans, there is increasing evidence that they are also valuable sources of phytosterols, including sitosterols. Several studies have characterized the biological activities of these sterols derived from *Agave*, highlighting their potential in functional food and pharmaceutical applications.

To better understand the methodologies available for isolating BSS from *Agave* species and other plant matrices, a comparative overview is presented of the most employed solvent-based extraction techniques (Table 3). This table includes both conventional and modern approaches, highlighting key operational parameters, solvent types, plant sources, advantages, and limitations. While some methods such as Soxhlet extraction and maceration have been directly applied to *Agave* species, others—such as supercritical CO_2 extraction—have been more extensively explored in different matrices yet offer promising alternatives for future application to *Agave*. Notably, MAE has demonstrated superior performance in terms of yield and efficiency when extracting BSS from *A. angustifolia*. This comparative analysis underscores the need to balance efficiency, sustainability, and feasibility when selecting extraction methods, especially for bioactive compounds intended for food and pharmaceutical applications.

The evolution of extraction technologies reflects the growing interest in enhancing both the efficiency and sustainability of phytosterol recovery from plant matrices. While conventional methods such as Soxhlet and maceration remain widely used, their limitations—particularly high solvent consumption and prolonged extraction times—have prompted the development of greener alternatives. Among these, MAE stands out for its reduced environmental impact, greater selectivity, and improved extraction efficiency. Its successful application to *A. angustifolia* underscores its potential as a scalable and eco-friendly technique for isolating BSS and other bioactive compounds from *Agave*-derived residues.

Table 3. Comparative Table of Solvent Extraction Methods for Isolating β-Sitosterol from *Agave* spp. and Other Plant Sources

Extraction Method	Plant Matrix	Solvent(s)	Main Characteristics	Advantages	Limitations	References
Soxhlet Extraction	*Agave* spp.	n-Hexane, petroleum ether, ethanol	Continuous hot solvent circulation for exhaustive extraction	High efficiency; widely referenced in literature	Time-consuming; large volumes of hazardous solvents required	(Uddin *et al*. 2018; Chemat *et al*. 2020)
Maceration	*Agave* spp.	Ethanol, hexane	Powdered plant material soaked in solvent at room temperature with occasional stirring	Simple, low-cost, accessible	Long extraction time; less efficient at ambient temperature for low-solubility compounds	(Santiago-Martínez *et al*. 2023; Vilela *et al.* 2013)
Microwave-Assisted Extraction (MAE)	*Agave angustifolia*	Ethanol, aqueous ethanol	Microwave energy heats plant matrix and solvent; enhances yield of β-sitosterol	Higher yield of β-sitosterol; reduced time and solvent use; green chemistry compliant	Requires optimization of solvent and conditions	(López-Salazar *et al.* 2019; Brusotti *et al.* 2014).
Saponification + Solvent	Corn fiber, other plants	Ethanol + NaOH or KOH	Hydrolysis of esterified sterols to free form prior to analysis	Enables quantification of total and individual sterols	Requires high temperature and chemical reagents	(Rohr *et al*. 2005; Abbas *et al*. 2010)
Supercritical CO_2 Extraction (SFE)	Cocoa butter	CO_2 ± ethanol (cosolvent	Extraction under high pressure and temperature	High purity extracts; minimal solvent residues	Requires specialized equipment; not yet reported for *Agave sitosterols*	(Roiaini *et al*. 2016)

Biological Activity of Sitosterol from Different *Agave* Plants

The biological activity of BSS and BSSG derived from various *Agave* species has garnered increasing attention due to their pharmacological potential. Several studies have highlighted their antimicrobial, anti-inflammatory, antioxidant, cytoprotective, and immunomodulatory properties, particularly *in vitro* and in *vivo* models.

In a different study, the extraction BSS and BSSG from *A. angustifolia* bagasse was carried out using MAE with ethanol as the solvent. Quantification and characterization of BSS and BSSG were performed using high-performance thin layer chromatography (HPTLC), Fourier transform infrared spectroscopy (FT-IR), high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), and gas chromatography-mass spectrometry (GC-MS). With an extraction time of 9 seconds, MAE yielded a higher amount of BSS (103.6 mg/g dry weight) compared to BSSG (61.6 mg/g dry weight). This study highlights MAE as an efficient method to recover BSS and BSSG from *A. angustifolia* bagasse and supports the sustainable valorization of *Agave* industry by-products, aligning with green and circular economy principles (López-Salazar *et al.* 2024).

One of the most notable applications of BSS and BSSG is in wound healing and cytoprotection. Extracts from *A. angustifolia* bagasse obtained through MAE have been shown to enhance fibroblast viability and proliferation. *In vitro* studies using HDFn (human dermal fibroblasts isolated from the foreskin of a newborn) demonstrated that short MAE extraction times (*e.g.*, 5 to 9 seconds) with ethanol as solvent resulted in high BSS content (up to 103.6 mg/g dry weight), with no cytotoxicity even at elevated concentrations. Notably, fibroblast proliferation increased by up to 24% with 500 µg/mL of extract, indicating biocompatibility and potential to stimulate tissue regeneration (Chu-Martínez, 2024; López-Salazar *et al*. 2022b).

In addition, anti-inflammatory activity has been demonstrated in both *in vitro* and *in vivo* models. In a TPA-induced ear edema model, ethanolic extracts of *A. angustifolia* reduced inflammation by 74% at low doses (3 mg/ear), an effect attributed to the presence of BSSG and potentially other minor compounds (López-Salazar *et al*. 2022a). Hernández-Valle *et al*. (2014) identified 3-O-palmitoyl-glucopyranosyl sitosterol as a key active component, underscoring the role of sitosterol derivatives in modulating inflammatory responses via cytokine regulation.

The immunomodulatory effects of *Agave*-derived extracts have also been documented in models of systemic autoimmune disease. In mice with pristane-induced systemic lupus erythematosus (SLE), treatment with *A. tequilana* acetone extracts and fructans significantly reduced pathological markers, including joint inflammation, proteinuria, and pro-inflammatory cytokines (Gutiérrez Nava *et al.* 2017). These effects are likely mediated by BSSG, phytol, and other sterols identified through phytochemical analyses.

Regarding antimicrobial activity, comparative studies of *A. tequilana, A. angustifolia, A. rhodacantha,* and *A. maximiliana* revealed species-specific amoebicidal effects against *E. histolytica*. *A. tequilana* exhibited the highest potency, achieving over 90% trophozoite inhibition at concentrations of 300 to 600 µg/Ml. This is likely due to its rich content of flavonoids (quercetin, kaempferol), gallic acid, BSS, and saponins. In contrast, *A. angustifolia* showed moderate activity, while other species demonstrated limited effects (Rodríguez-Zapata *et al*. 2024). These findings highlight the variation in secondary metabolite profiles among *Agave* species and the importance of targeted phytochemical characterization in pharmacological assessments.

Among extraction methodologies, MAE has proven superior to conventional methods such as maceration in both efficiency and yield. For instance, a 5-second MAE extraction from *A. angustifolia* produced approximately 125 mg/g of BSSG, whereas conventional maceration yielded only 26.7 mg/g, underscoring MAE as a rapid and sustainable alternative aligned with circular economy principles (López-Salazar *et al*. 2019). Furthermore, the use of potassium hydroxide as a catalyst during extraction, particularly from mezcal industry bagasse, significantly enhanced BSS and BSSG yields (García-Ávila *et al*. 2022), offering a value-added application for agro-industrial residues.

The antioxidant and antihypertensive potential of *Agave* extracts has also been reported. In a murine model of angiotensin II-induced systemic arterial hypertension, crude acetone extract and ethyl acetate fractions from *A. tequilana* leaves lowered blood pressure and modulated cytokine levels, including IL-1β, IL-6, and TNF-α. These effects were associated with the presence of phytosterols and fatty acids such as phytol and 9,12-octadecadienoic acid (Herrera-Ruiz *et al*. 2022), suggesting a synergistic mechanism of action. Recent findings have further confirmed the regenerative potential of microwave-assisted *A. angustifolia* bagasse extracts. In a murine excision wound model, López-Salazar *et al*. (2025) demonstrated that a topical application of 8 mg of BagEE (Bagasse Ethanolic Extract) significantly accelerated wound healing. By day 13, the treatment of wounds showed 99.4% closure compared to 92.8% closure in the control group by day 22. Complete re-epithelialization and organized skin structure were observed, suggesting potent wound-healing properties. HPLC-MS analysis identified key compounds including quercetin, isorhamnetin, diosgenin, hecogenin, manogenin, BSSG, and BSS, indicating a complex phytochemical composition contributing to the observed biological effects.

Collectively, these findings underscore the therapeutic promise of sitosterols and their derivatives from *Agave* species. Their multifaceted bioactivities ranging from tissue regeneration and inflammation control to immune modulation and antimicrobial effects, highlight their potential for the development of plant-based interventions.

This synthesis reinforces the relevance of *Agave*-derived extracts as multifunctional agents in the development of natural therapeutic products. However, further research is required to establish standardized extraction protocols, achieve in-depth characterization of active metabolites, and evaluate their efficacy in controlled clinical settings. As summarized in Table 4, different *Agave* species exhibit varying levels of BSS and its glucoside, which are extracted using distinct methodologies, including MAE and maceration. These compounds have been associated with diverse biological activities, such as promoting cell viability, anti-inflammatory effects, and mitigating chronic hypertension. The data presented in the table underscore the importance of extraction methods and plant parts used in obtaining bioactive compounds, reinforcing the relevance of *Agave*-derived products in therapeutic applications. Such studies will lay the foundation for the rational use of *Agave* resources in evidence-based phytomedicine and wound care applications.

Table 4. Sitosterols from Different *Agave* Plants

***Agave* plant**	**Part of the *Agave* used**	**Extraction Method**	**Type of extract**	**Sitosterol**	**Biological activity**	**References**
A. angustifolia	Bagasse	MAE	Ethanolic	BSS, BSSG	Wound healing in a murine model	(López-Salazar et al. 2025)
A. angustifolia	Bagasse	MAE	Ethanolic with KOH	BSS	Cell viability	(Chu-Martínez 2024)
A. tequilana, A. angustifolia, A. rhodacantha A. maximiliana	Leaves	The samples were incubated at 150 rpm for 48 hours at room temperature.	Ethanolic	BSS	Cytotoxic effect on *E. histolytica* trophozoites	(Rodríguez-Zapata *et al*. 2024).
A. angustifolia	Bagasse	MAE	Ethanolic	BSS, BSSG	---	(López-Salazar *et al*. 2024)
A. tequilana	Leaves	Maceration	Acetonic	t-sitosterol	Mitigates chronic hypertension and vascular damage	(Herrera-Ruiz *et al*. 2022)
A. angustifolia	Stem	MAE	Ethanolic	BSS, BSSG	Fibroblast proliferation *in vitro*. Non-cytotoxic	(López-Salazar *et al*. 2022b)
A. angustifolia	Bagasse	MAE	Ethanolic with KOH	BSS, BSSG	-----	(García-Ávila 2022)
A. angustifolia	Stem	MAE	Ethanolic	BSSG	Anti-Inflammatory effect	(López-Salazar 2022a)
A. angustifolia	Stem	MAE	Ethanolic with KOH	BSSG	--	(López-Salazar *et al*. 2019)
A. tequilana	Leaves	Maceration	Acetonic	BSSG	Immunomodulatory effect	(Gutiérrez *et al.*2017)
A. angustifolia	Stem	Maceration	Acetonic	3-O-[(6'-O-Palmitoyl)-β-D-glucopyranosyl Sitosterol BSSG	Anti-Inflammatory effect	(Hernández-Valle *et al*. 2014)

* MAE (microwave-assisted extraction), BSS (β-sitosterol) and BSSG (β-sitosterol β-d-glucoside).

CONCLUSIONS

This review highlights the pharmacological relevance of β-sitosterol (BSS) and β-sitosterol β-d-glucoside (BSSG) derived from various *Agave* species, emphasizing their wound healing, anti-inflammatory, antioxidant, and immunomodulatory properties. Empirical studies, particularly those using extracts from *A. angustifolia* and *A. tequilana*, demonstrate enhanced fibroblast viability, reduced inflammatory responses, and significant tissue regeneration in both *in vitro* and *in vivo* models. These biological effects are strongly associated with the presence of BSS, BSSG, and other co-occurring phytochemicals such as flavonoids and saponins.

Among the extraction techniques evaluated, microwave-assisted extraction (MAE) stands out for its efficiency in yielding high levels of BSS and BSSG in short time frames, confirming its potential as a sustainable alternative to conventional methods. Additionally, the topical application of bagasse-derived extracts has shown significant acceleration of wound closure and tissue remodeling in murine models, reinforcing the therapeutic potential of these compounds.

However, the extraction of sitosterols from *Agave* using green technologies such as supercritical fluid extraction (SFE) remains underexplored. Given the promising *in vitro* and *in vivo* outcomes, future research should prioritize optimizing environmentally friendly extraction protocols, standardizing active compound content and validating these findings in clinical settings. Such efforts will contribute to the rational development of *Agave*-based therapeutic agents and align with circular economy principles by valorizing agro-industrial byproducts.

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REFERENCES CITED

Abbas, C., Beery, K. E., Binder, T. P., and Rammelsberg, A. M. (2010). "Ethanol extraction of phytosterols from corn fiber," U.S. Patent No. 7,833,994.

Alcazar-Valle, M., Gschaedler, A., Gutierrez-Pulido, H., Arana-Sanchez, A., and Arellano-Plaza, M. (2019). "Fermentative capabilities of native yeast strains grown on juices from different *Agave* species used for tequila and mezcal production," *Brazilian Journal of Microbiology* 50, 379-388. DOI: 10.1007/s42770-019-00049-7

Alducin-Martínez, C., Ruiz Mondragón, K. Y., Jiménez-Barrón, O., Aguirre-Planter, E., Gasca-Pineda, J., Eguiarte, L. E., and Medellin, R. A. (2022). "Uses, knowledge and extinction risk faced by *Agave* species in Mexico," *Plants* 12(1), 124.14. DOI: 10.3390/plants12010124

Almaraz-Abarca, N., Delgado-Alvarado, E. A., Antonio'Avila-Reyes, J., Uribe-Soto, J. N., and González-Valdez, L. S. (2013). "The phenols of the genus *Agave*

(Agavaceae)" *Journal of Biomaterials and Nanobiotechnology* 2013, 4, 9-16. DOI: 10.4236/jbnb.2013.43A002

Álvarez-Chávez, J., Villamiel, M., Santos-Zea, L., and Ramírez-Jiménez, A. K. (2021). "*Agave* by-products: An overview of their nutraceutical value, current applications, and processing methods," *Polysaccharides* 2(3), 720-743. DOI: 10.3390/polysaccharides2030044

Álvarez-Ríos, G. D., Pacheco-Torres, F., Figueredo-Urbina, C. J., and Casas, A. (2020). "Management, morphological and genetic diversity of domesticated agaves in Michoacán, México," *Journal of Ethnobiology and Ethnomedicine* 16, 1-17. DOI: 10.1186/s13002-020-0353-9

Apolinario, A. C., de Carvalho, E. M., de Lima Damasceno, B. P. G., da Silva, P. C. D., Converti, A., Pessoa Jr, A., and da Silva, J. A. (2017). "Extraction, isolation and characterization of inulin from *Agave sisalana* boles," *Industrial Crops and Products* 108, 355-362. DOI: 10.1016/j.indcrop.2017.06.045

Arellano-Plaza, M., Paez-Lerma, J. B., Soto-Cruz, N. O., Kirchmayr, M. R., and Gschaedler Mathis, A. (2022). "Mezcal production in Mexico: Between tradition and commercial exploitation," *Frontiers in Sustainable Food Systems* 6, article 832532. DOI: 10.3389/fsufs.2022.832532

Awad, A. B., Chen, Y. C., Fink, C. S., and Hennessey, T. (1996). "Beta-sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids," *Anticancer Research* 16(5A), 2797-2804.

Beck, J. G., Mathieu, D., Loudet, C., Buchoux, S., and Dufourc, E. J. (2007). "Plant sterols in 'rafts': A better way to regulate membrane thermal shocks," *The FASEB Journal* 21(8), 1714-1723. DOI: 10.1096/fj.06-7809com

Blas-Yañez, S., and Thomé-Ortiz, H. (2021). "Agave pulquero (*Agave salmiana*), socio-economic and agro-ecological importance and its development perspectives: A literature review," *Ciência Rural* 51, article e20200441. DOI: 10.1590/0103-8478cr20200441

Brusotti, G., Cesari, I., Dentamaro, A., Caccialanza, G., and Massolini, G. (2014). "Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethnopharmacological approach," *Journal of Pharmaceutical and Biomedical Analysis* 87, 218-228. DOI: 10.1016/j.jpba.2013.03.007

Cao, Z. Q., Wang, X. X., Lu, L., Xu, J. W., Li, X. B., Zhang, G. R., and Song, Y. J. (2019). "β-Sitosterol and gemcitabine exhibit synergistic anti-pancreatic Cancer activity by modulating apoptosis and inhibiting epithelial–mesenchymal transition by deactivating Akt/GSK-3β signaling," *Frontiers in Pharmacology* 9, article 1525. DOI: 10.3389/fphar.2018.01525

Chai, J. W., Kuppusamy, U. R., and Kanthimathi, M. S. (2008). "Beta-sitosterol induces apoptosis in MCF-7 cells," *Malaysian Journal of Biochemistry and Molecular Biology* 16(2), 28-30.

Chanioti, S., Katsouli, M., and Tzia, C. (2021). "β-Sitosterol as a functional bioactive," in: *A Centum of Valuable Plant Bioactives*, Academic Press, pp. 193-212. DOI: 10.1016/B978-0-12-822923-1.00014-5

Chemat, F., Vian, M. A., Fabiano-Tixier, A. S., Nutrizio, M., Jambrak, A. R., Munekata, P. E., and Cravotto, G. (2020). "A review of sustainable and intensified techniques for extraction of food and natural products," *Green Chemistry* 22(8), 2325-2353. DOI: 10.1039/C9GC03878G

Chen, S., Wang, R., Cheng, M., Wei, G., Du, Y., Fan, Y., and Deng, Z. (2020). "Serum

cholesterol-lowering activity of β-sitosterol laurate is attributed to the reduction of both cholesterol absorption and bile acids reabsorption in hamsters," *Journal of Agricultural and Food Chemistry* 68(37), 10003-10014. DOI: 10.1021/acs.jafc.0c03917

Chu Martínez, A. (2024). "Efecto de extractos de residuos de *Agave angustifolia* Haw sobre la viabilidad in vitro de células involucras en procesos de cicatrización," Tesis de maestría no publivada, Instituto Politécnico Nacional.

Colunga-GarcíaMarín, P., Zizumbo-Villarreal, D., and Martínez-Torres, J. (2007). "Tradiciones en el aprovechamiento de los agaves Mexicanos: Una aportación a la protección legal y conservación de su diversidad biológica y cultural," in*: Lo Ancestral Hay Futuro: Del Tequila, los Mezcales y Otros Agaves*, 248(5).

Consejo Regulador del Tequila (Tequila Regulatory Council), CRT. (2022). "Información estadística. Producción de tequila" (database). https://www.crt.org.mx/EstadisticasCRTweb/

Cui, S., Jiang, H., Chen, L., Xu, J., Sun, W., Sun, H., amd Qu, W. (2020). "Design, synthesis and evaluation of wound healing activity for β-sitosterols derivatives as potent Na^+/K^+-ATPase inhibitors," *Bioorganic Chemistry* 98, article 103150. DOI: 10.1016/j.bioorg.2019.103150

Davis, S. C., and Ortiz-Cano, H. G. (2023). "Lessons from the history of *Agave*: Ecological and cultural context for valuation of CAM," *Annals of Botany* 132(4), 819-833. DOI: 10.1093/aob/mcad072

Díaz Castellanos, R. (2023). "The price of agave in Mexican states holding Designation of Origin of Tequila status, and its systematic depreciation over the forthcoming decade (2000-2031)," *The Anáhuac Journal* 23(2), 12-37. DOI: 10.36105/theanahuacjour.2023v23n2.01

Espinosa-Andrews, H., Urias-Silvas, J. E., and Morales-Hernandez, N. (2021). "The role of *Agave fructans* in health and food applications: A review," *Trends in Food Science & Technology*, 114, 585-598. DOI: 10.1016/j.tifs.2021.06.022

Estrada-Maya, A., and Weber, B. (2022). "Biogás y bioetanol a partir de bagazo de agave sometido a explosión de vapor e hidrólisis enzimática," *Ingeniería, Investigación y Tecnología* 23(2), article 009. DOI: 10.22201/fi.25940732e.2022.23.2.009

Ferrer, A., Altabella, T., Arró, M., and Boronat, A. (2017). "Emerging roles for conjugated sterols in plants," *Progress in Lipid Research* 67, 27-37. DOI: 10.1016/j.plipres.2017.06.002

García-Ávila, E. E., Arenas-Ocampo, M., and Camacho-Díaz, B. (2022). *Obtención de Fitoesteroles de Bagazo Residual de Agave angustifolia Haw con Extracción Asistida por Microondas*, Master's Thesis, Instituto Politécnico Nacional.

García-Mendoza, A. J., Franco Martínez, I. S., and Sandoval Gutiérrez, D. (2019). "Cuatro especies nuevas de Agave (Asparagaceae, Agavoideae) del sur de México," *Acta botánica mexicana* 126.

García-Morales, S., Corzo-Jiménez, I. J., Silva-Córdova, N. F., Soto-Cordero, A. M., Rodríguez-Mejía, D. I., Pardo-Núñez, J., and León-Morales, J. M. (2022). "Comparative study of steroidal sapogenins content in leaves of five *Agave* species," *Journal of the Science of Food and Agriculture* 102(13), 5653-5659. DOI: 10.1002/jsfa.11912

Gupta, A., Naraniwal, M., and Kothari, V. (2012). "Modern extraction methods for preparation of bioactive plant extracts," *International Journal of Applied and Natural Sciences* 1(1), 8-26.

Gutiérrez Nava, Z. J., Jiménez-Aparicio, A. R., Herrera-Ruiz, M. L., and Jiménez-Ferrer, E. (2017). "Immunomodulatory effect of *Agave tequilana* evaluated on an autoimmunity like-SLE model induced in Balb/c mice with pristane," *Molecules* 22(6), 848. DOI: 10.3390/molecules22060848

Hernández-Valle, E., Herrera-Ruiz, M., Rosas Salgado, G., Zamilpa, A., Arenas Ocampo, M. L., Jiménez Aparicio, A., ... and Jiménez-Ferrer, E. (2014). "Antiinflammatory effect of 3-O-[(6'-O-palmitoyl)-β-D-glucopyranosyl sitosterol] from *Agave angustifolia* on ear edema in mice," *Molecules* 19(10), 15624-15637. DOI: 10.3390/molecules191015624

Herrera-Ruiz, M., Gutiérrez-Nava, Z. J., Trejo-Moreno, C., Zamilpa, A., González-Cortazar, M., Jiménez-Aparicio, A. R., and Jiménez-Ferrer, E. (2022). "*Agave tequilana* counteracts chronic hypertension and associated vascular damage," *Journal of Medicinal Food* 25(4), 443-455. DOI: 10.1089/jmf.2021.0044

Jourdain, C., Tenca, G., Deguercy, A., Troplin, P., and Poelman, D. (2006). "*In-vitro* effects of polyphenols from cocoa and β-sitosterol on the growth of human prostate cancer and normal cells," *European Journal of Cancer Prevention* 15(4), 353-361.

Kartosentono, S., Suryawati, S., Indrayanto, G., and Zaini, N. C. (2002). "Accumulation of Cd^{2+} and Pb^{2+} in the suspension cultures of *Agave amaniensis* and *Costus speciosus* and the determination of the culture's growth and phytosteroid content," *Biotechnology Letters* 24, 687-690. DOI: 10.1023/A:1015225931409

Kongduang, D., Wungsintaweekul, J., and De-Eknamkul, W. (2008). "Biosynthesis of β-sitosterol and stigmasterol proceeds exclusively *via* the mevalonate pathway in cell suspension cultures of *Croton stellatopilosus*," *Tetrahedron Letters* 49(25), 4067-4072. DOI: 10.1016/j.tetlet.2008.04.049

Lim, T. K. (2012). *Edible Medicinal and Non-medicinal Plants*, Springer, Dordrecht, The Netherland.

López Salazar, H. (2022a). *Actividad Antiinflamatoria de un Extracto Estandarizado de Glucósido de β-sitosterol de Agave angustifolia Haw Obtenido por Extracción Asistida por Microondas*, Ph.D. Dissertation, Instituto Politécnico Nacional.

López-Salazar, H., Camacho-Díaz, B. H., Ávila-Reyes, S. V., Pérez-García, M. D., González-Cortazar, M., Arenas Ocampo, M. L., and Jiménez-Aparicio, A. R. (2019). "Identification and quantification of β-sitosterol β-D-glucoside of an ethanolic extract obtained by microwave-assisted extraction from *Agave angustifolia* Haw," *Molecules* 24(21), article 3926. DOI: 10.3390/molecules24213926

López-Salazar, H., Camacho-Díaz, B. H., Ocampo, M. L. A., and Jiménez-Aparicio, A. R. (2023) "Microwave-assisted extraction of functional compounds from plants: A review," *BioResources* 18(3), 6614-6638. DOI: 10.15376/biores.18.3.6614-6638

López-Salazar, H., Hildeliza Camacho-Díaz, B., Arenas Ocampo, M. L., Campos-Mendiola, R., Martínez-Velarde, R., López-Bonilla, A., and Ruperto Jiménez-Aparicio, A. (2024). "Microwave-assisted extraction of β-sitosterol: A by-product from *Agave angustifolia* Haw bagasse," *BioResources* 19(1), 568-581. DOI: 10.15376/biores.19.1.568-581

López-Salazar, H., Negrete-León, E., Camacho-Díaz, B. H., Acevedo-Fernández, J. J., Ávila-Reyes, S. V., and Ocampo, M. L. A. (2025). "The effect of *Agave* bagasse extract on wound healing in a murine model," *Future Pharmacology* 5(1), article 0008. DOI: 10.3390/futurepharmacol5010008

López-Salazar, H., Tapia, J. S. O., Camacho-Díaz, B. H., Ocampo, M. L. A., and Jiménez-Aparicio, A. R. (2022b). "Evaluation of biocompatibility of a standardized

extract of *Agave angustifolia* Haw in human dermal fibroblasts," in: *Recent Trends in Sustainable Engineering: Proceedings of the 2nd International Conference on Applied Science and Advanced Technology*, Springer International Publishing, pp. 107-116.

Márquez-Rangel, I., Cruz, M., Ruiz, H. A., Rodríguez-Jasso, R. M., Loredo, A., and Belmares, R. (2023). "*Agave* waste as a source of prebiotic polymers: Technological applications in food and their beneficial health effect," *Food Bioscience* 2023, 103102. DOI: 10.1016/j.fbio.2023.103102

Morales-Serna, J. A., Jiménez, A., Estrada-Reyes, R., Marquez, C., Cárdenas, J., and Salmón, M. (2010). "Homoisoflavanones from *Agave tequilana* weber," *Molecules* 15(5), 3295-3301. DOI: 10.3390/molecules15053295

Morán-Velázquez, D. C., Monribot-Villanueva, J. L., Bourdon, M., Tang, J. Z., López-Rosas, I., Maceda-López, L. F., Villalpando-Aguilar, J. L., Rodríguez-López, L., Gauthier, A., Trejo, L., *et al*. (2020). "Unravelling chemical composition of Agave spines: News from *Agave fourcroydes* Lem," *Plants* 9(12), article 1642. DOI: 10.3390/plants9121642

Moreau, R. A., Whitaker, B. D., and Hicks, K. B. (2002). "Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses," *Progress in Lipid Research* 41(6), 457-500. DOI: 10.1016/S0163-7827(02)00006-1

Moreno-Anguiano, O., Cloutier, A., Rutiaga-Quiñones, J. G., Wehenkel, C., Rosales-Serna, R., Rebolledo, P., Hernández-Pacheco, C. E., and Carrillo-Parra, A. (2022). "Use of *Agave durangensis* bagasse fibers in the production of wood-based medium density fiberboard (MDF)," *Forests* 13(2), article 271. DOI: 10.3390/f13020271

Morreeuw, Z. P., Castillo-Quiroz, D., Ríos-González, L. J., Martínez-Rincón, R., Estrada, N., Melchor-Martínez, E. M., and Reyes, A. G. (2021). "High throughput profiling of flavonoid abundance in *Agave lechuguilla* residue-valorizing under explored mexican plant," *Plants* 10(4), article 695. DOI: 10.3390/plants10040695

Nava-Cruz, N. Y., Medina-Morales, M. A., Martinez, J. L., Rodriguez, R., and Aguilar, C. N. (2015). "*Agave* biotechnology: An overview," *Critical Reviews in Biotechnology* 35(4), 546-559. DOI: 10.3109/07388551.2014.923813

Nirmal, S. A., Pal, S. C., Mandal, S. C., and Patil, A. N. (2012). "Analgesic and anti-inflammatory activity of β-sitosterol isolated from *Nyctanthes arbortristis* leaves," *Inflammopharmacology* 20, 219-224. DOI: 10.1007/s10787-011-0110-8

Ogbe, R. J., Ochalefu, D. O., Mafulul, S. G., and Olaniru, O. B. (2015). "A review on dietary phytosterols: Their occurrence, metabolism and health benefits," *Asian J. Plant Sci. Res*. 5(4), 10-21.

Paniagua-Pérez, R., Flores-Mondragón, G., Reyes-Legorreta, C., Herrera-López, B., Cervantes-Hernández, I., Madrigal-Santillán, O., and Madrigal-Bujaidar, E. (2017). "Evaluation of the anti-inflammatory capacity of beta-sitosterol in rodent assays," *African Journal of Traditional, Complementary and Alternative Medicines* 14(1), 123-130.

Pérez-Zavala, M. D. L., Hernández-Arzaba, J. C., Bideshi, D. K., and Barboza-Corona, J. E. (2020). "*Agave*: A natural renewable resource with multiple applications," *Journal of the Science of Food and Agriculture* 100(15), 5324-5333. DOI: 10.1002/jsfa.10586

Phatangare, N. D., Deshmukh, K. K., Murade, V. D., Naikwadi, P. H., Hase, D. P., Chavhan, M. J., and Velis, H. E. (2017). "Isolation and characterization of β-sitosterol from *Justicia gendarussa* burm. F. – An anti-inflammatory compound," *Int. J. Pharmacogn. Phytochem. Res*. 9(9), 1280-1287.

Ramírez-Guzmán, K. N., Torres-León, C., Martinez-Medina, G. A., de la Rosa, O., Hernández-Almanza, A., Alvarez-Perez, O. B., and Aguilar, C. N. (2019). "Traditional fermented beverages in Mexico," in: *Fermented beverages*, Woodhead Publishing, pp. 605-635.

Rizwan, K., Zubair, M., Rasool, N., Riaz, M., Zia-Ul-Haq, M., and De Feo, V. (2012). "Phytochemical and biological studies of *Agave attenuate*," *International Journal of Molecular Sciences* 13(5), 6440-6451. DOI: 10.3390/ijms13056440

Rodríguez-Zapata, A. L., Mora-Frias, J. I., Briano-Elias, M. A., Pérez-Centeno, A., Barrientos-Ramírez, L., Reynoso-Orozco, R., and Castillo-Romero, A. (2024). "Phytochemical analysis and amoebicidal evaluation of different *Agave* species," *Applied Sciences* 14(5), article 1905. DOI: 10.3390/app14051905

Rohr, R., Rohr, R., and Trujillo-Quijano, J. A. (2005). "Process for separating unsaponifiable valuable products from raw materials," U.S. Patent No. 6,846,941.

Roiaini, M., Seyed, H. M., Jinap, S., and Norhayati, H. (2016). "Effect of extraction methods on yield, oxidative value, phytosterols and antioxidant content of cocoa butter," *International Food Research Journal* 23(1), article 47.

Ruiz, H. A., Martínez, A., and Vermerris, W. (2016). "Bioenergy potential, energy crops, and biofuel production in Mexico," *BioEnergy Research* 9, 981-984. DOI: 10.1007/s12155-016-9802-7

Sabater, C., Calvete-Torre, I., Villamiel, M., Moreno, F. J., Margolles, A., and Ruiz, L. (2021). "Vegetable waste and by-products to feed a healthy gut microbiota: Current evidence, machine learning and computational tools to design novel microbiome-targeted foods," *Trends in Food Science & Technology* 118, 399-417. DOI: 10.1016/j.tifs.2021.10.002

Saeidnia, S., Manayi, A., Gohari, A. R., and Abdollahi, M. (2014). "The story of beta-sitosterol – A review," *European Journal of Medicinal Plants* 4(5), 590-609.

Santiago-Martínez, A., Pérez-Herrera, A., Martínez-Gutiérrez, G. A., and Meneses, M. E. (2023). "Contributions of agaves to human health and nutrition," *Food Bioscience* 53, article 102753. DOI: 10.1016/j.fbio.2023.102753

Seidel, V. (2005). "Initial and bulk extraction," *Natural Products Isolation*, 27-46.

Sidana, J., Singh, B., and Sharma, O. P. (2016). "Saponins of *Agave*: Chemistry and bioactivity," *Phytochemistry* 130, 22-46. DOI: 10.1016/j. phytochem.2016.06.010

Smith, R. M. (2003). "Before the injection—Modern methods of sample preparation for separation techniques," *Journal of Chromatography A* 1000(1-2), 3-27. DOI: 10.1016/S0021-9673(03)00511-9

Soto-Castro, D., Pérez-Herrera, A., García-Sánchez, E., and Santiago-García, P. A. (2021). "Identification and quantification of bioactive compounds in *Agave potatorum* Zucc. leaves at different stages of development and a preliminary biological assay," *Waste and Biomass Valorization* 12, 4537-4547. DOI: 10.1007/s12649-020-01329-2

Šovljanski, O., Travičić, V., Tomić, A., Vulić, J., Šaponjac, V. T., Ćetković, G., and Čanadanović-Brunet, J. (2023). "From agricultural waste to functional food products: An overview," in: *Agricultural Waste: Environmental Impact, Useful Metabolites and Energy Production*, pp. 489-520. DOI: 10.1007/978-981-19-8774-8_18

Stewart, J. R. (2015). "*Agave* as a model CAM crop system for a warming and drying world," *Frontiers in Plant Science* 6, article 684. DOI: 10.3389/fpls.2015.00684

Suárez-González, E. M., López, M. G., Délano-Frier, J. P., and Gómez-Leyva, J. F. (2014). "Expression of the 1-SST and 1-FFT genes and consequent fructan

accumulation in *Agave tequilana* and *A. inaequidens* is differentially induced by diverse (a) biotic-stress related elicitors," *Journal of Plant Physiology* 171(3-4), 359-372. DOI: 10.1016/j.jplph.2013.08.002

Sun, Y., Liu, X., and Pian, G. (2019). "Effect and mechanism study on the autophagy and apoptosis induced by β-sitosterol in human gastric cancer cells," *Journal of Chinese Physician* 866-871.

Torres-García, I., Rendón-Sandoval, F. J., Blancas, J., and Moreno-Calles, A. I. (2019). "The genus *Agave* in agroforestry systems of Mexico," *Botanical Sciences* 97(3), 263-290. DOI: 10.17129/botsci.2202D

Uddin, M. S., Ferdosh, S., Haque Akanda, M. J., Ghafoor, K., Rukshana, A. H., Ali, M. E., Kamaruzzaman, B. Y., Fauzi M. B., Hadijah, S., Shaarani, S., and Islam Sarker, M. Z. (2018). "Techniques for the extraction of phytosterols and their benefits in human health: A review," *Separation Science and Technology* 53(14), 2206-2223. DOI: 10.1080/01496395.2018.1454472

Vilela, C., Santos, S. A., Oliveira, L., Camacho, J. F., Cordeiro, N., Freire, C. S., and Silvestre, A. J. (2013). "The ripe pulp of *Mangifera indica* L.: A rich source of phytosterols and other lipophilic phytochemicals," *Food Research International* 54(2), 1535-1540. DOI: 10.1016/j.foodres.2013.09.017

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