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GC-MS profiling of bioactive compounds in methanolic extract of *Aloe barbadensis* Mill. and their therapeutic potential

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Abstract

This study examined the phytochemical compounds found in a methanolic extract of *Aloe barbadensis* Mill. leaves through the use of GC-MS. The researchers aimed to profile these compounds and discuss their potential therapeutic applications, given *Aloe vera* (*A. vera*) widespread use in traditional medicine. Fresh aloe leaves were harvested, dried, powdered, and extracted using methanol in a Soxhlet apparatus. The extract was analyzed with Gas chromatography and mass spectroscopy (GC-MS), with compounds identified by matching their mass spectra to the NIST library. The examination uncovered various important bioactive chemicals, such as β-sitosterol (14.41%), n-hexadecanoic acid (2.33%), 2-furancarboxaldehyde, 5-(hydroxymethyl)- (5.87%), and squalene (2.93%). These compounds are known to possess different advantageous properties, including anti-inflammatory, anticancer, and antioxidant effects. The discovery of these substances offers scientific backing for the traditional benefits of *A. vera* and showcases its potential medical uses.

1. Introduction

Medicinal plants play a significant role in phytochemistry (Pankaj *et al*., 2022). *Aloe vera* (*Aloe barbadensis* Miller), a plant with extensive medicinal properties has been commonly used in traditional medicine due to its healing qualities (Sánchez *et al*., 2020). The gel and latex of the plant contain different bioactive compounds like polysaccharides, glycoproteins, and small molecules, which play a role in its pharmaceutical effects (Maan *et al*., 2018). Phytochemicals with medicinal benefits are beneficial presents for human well-being (Ankita *et al*., 2021). *A. vera* is widely recognized for its advantageous characteristics like antiviral, antioxidant, antibacterial, anti-inflammatory, laxative, anticancer, antidiabetic, antiallergic, immunostimulant, UV protection, and others (Vaidya *et al*., 2021). However, the lack of standardization and quality control in herbal medicine has hindered the integration of *A. vera* products into conventional healthcare (Guo and Mei, 2016). Although, *A. vera* has been used for a long time, more thorough scientific research is required to completely understand how its bioactive compounds work and to establish strong evidence of its effectiveness in treating different health conditions (Choche *et al*., 2014). The goal of this research was to thoroughly analyze the bioactive substances found in a methanolic extract of *A. vera* leaves using GC-MS, explore their potential for therapy, and emphasize the significance of standardized and evidence-based methods in phytotherapy.

Figure 1: ***Aloe barbadensis*** **Mill. plant.**

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2. Materials and Methods

2.1 Plant material and extract preparation

The plant material was authenticated by Botanical Survey of India, Coimbatore, India with Voucher Specimen number (BSI/SRC/8/16/2024-25/Tech-431). Fresh *Aloe barbadensis* Mill. leaves (Figure 1) were harvested from the experimental field at the Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University. The leaves were cleaned, air-dried, and then crushed into a fine powder. Soxhlet apparatus was used to extract 50 g of powdered sample with 500 ml methanol for 24 h. The solution was evaporated under low pressure and kept at 4°C until testing.

2.2 Gas chromatography-mass spectroscopy (GC-MS) analysis

The gas chromatography-mass spectroscopy (GC-MS) analysis was conducted using an Agilent 7890B gas chromatograph coupled with a 5977A mass spectrometer. Separation was achieved on a 30 m × 0.25 mm HP-5MS capillary column with 0.25 µm. The oven temperature was programmed to rise by 10°C per min. starting at 60°C and reaching 280°C. Helium was flowing as a carrier gas at a rate of 1 ml per min. The sample was injected using split mode (1:10) injection at a temperature of 250°C. Mass spectroscopy settings include scanning a range of 40-600 m/z and operating in EI mode at 70 eV.

2.3 Data processing and compound identification

Compounds were identified by comparing their retention indexes with known values in literature and comparing their mass spectra with the NIST 2017 library. Peak area percentages were employed for quantifying the samples.

3. Results

3.1 Major bioactive compounds identified

GC-MS examination of the methanolic extract showed the presence of various bioactive constituents. The major compounds were â-sitosterol (14.41%), 1-isopropoxy-2,2,3-trimethylaziridine (14.43%), n-hexadecanoic acid (2.33%), 2-furancarboxaldehyde, 5-(hydroxymethyl) (5.87%), and squalene (2.93%) (Table 1) (Figure1). Other notable compounds identified include phytol (1.13%), 9,12,15-octadecatrienoic acid (2.92%), lupeol (5.39%), and γ-tocopherol (0.65%). These compounds have numerous reported biological actions, which include, anti-inflammatory, and potential anticancer properties (Medeiros *et al*., 2007; Jain *et al*., 2016; López *et al*., 2013).

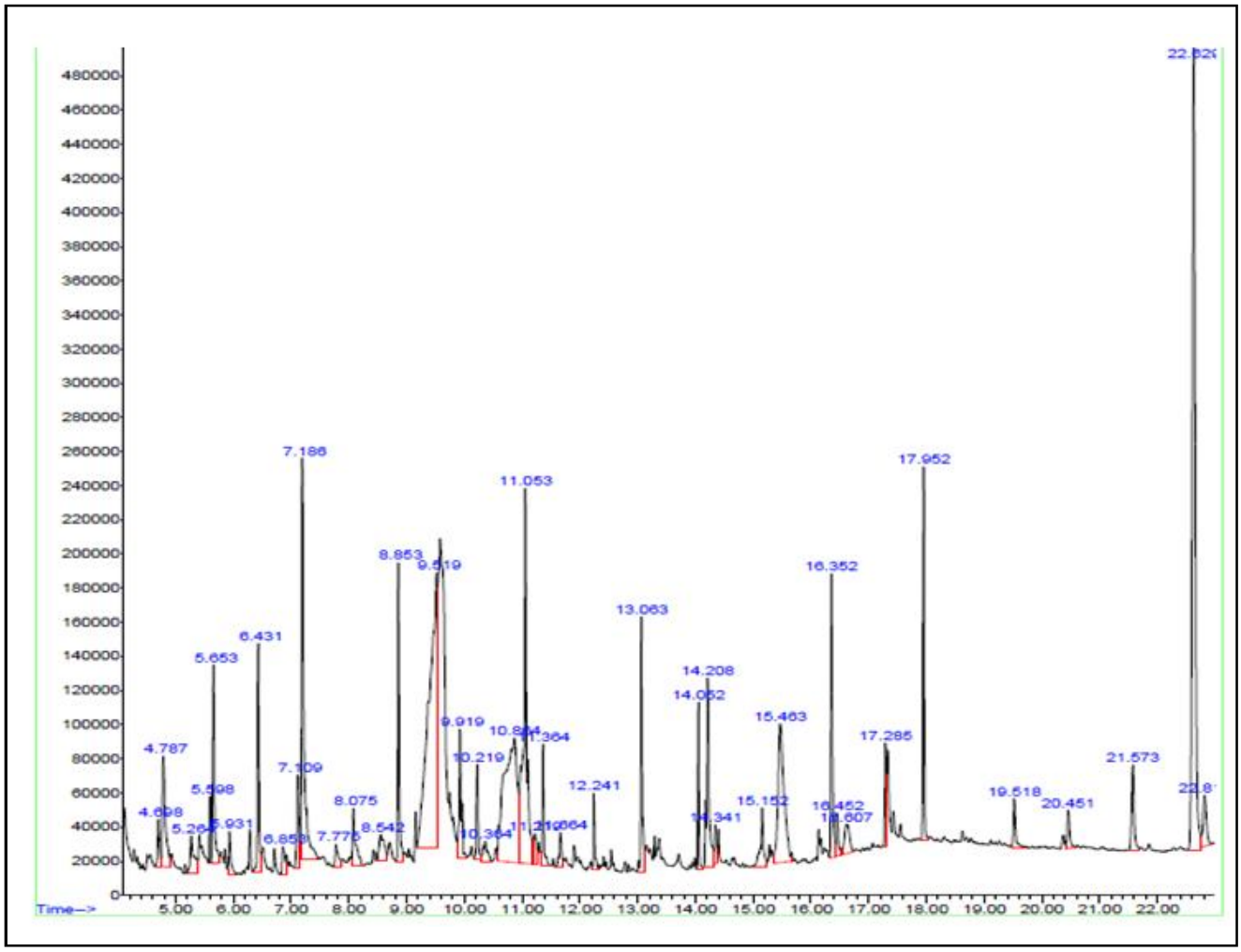

Figure 2: Gas chromatogram of *A. vera* methanolic extract.

Table 1: Major compounds identified in *A. vera* methanolic extract by GC-MS

Rank	Compound	Retention time (min)	Peak area (%)	Molecular weight (g/mol)	Biological activities
1.	β-sitosterol	22.629	14.41	414.71	Cholesterol-lowering, anticancer, immunomodulatory
2.	1-isopropoxy-2,2,3-trimethylaziridine	9.519	14.43	143.23	Potential antimicrobial, further investigation needed
3.	2-furancarboxaldehyde, 5-(hydroxymethyl)-	7.186	5.87	126.11	Antioxidant, anti-inflammatory, neuroprotective
4.	Lupeol	15.463	5.39	426.72	Anti-inflammatory, anticancer, antimicrobial
5.	Ar-tumerone	11.053	7.18	216.32	Anti-inflammatory, antioxidant
6.	Squalene	17.952	2.93	410.72	Antioxidant, anticancer, immune-enhancing
7.	9,12,15-octadecatrienoic acid	14.208	2.92	278.43	Anti-inflammatory, cardioprotective
8.	n-hexadecanoic acid	13.063	2.33	256.42	Antimicrobial, anti-inflammatory
9.	Hexadecanoic acid, 2,3-dihydroxypropyl ester	16.352	2.69	330.50	Emollient, skin conditioning
10.	Thymine	5.653	2.31	126.11	DNA/RNA component, potential antiviral

4. Discussion

The popularity of herbal medicine is on the rise, primarily due to its generally minimal adverse effects, enduring therapeutic benefits, and cost-effectiveness, despite potentially longer treatment periods. *A. vera* stands out as a remarkable example of nature's pharmacy, boasting a wide array of biological activities including antioxidant, antimicrobial, and anti-inflammatory properties. The extraction and analysis of this plant's phytoconstituents, coupled with the evaluation of their pharmacological potential, are crucial steps in modern drug development processes. Notably, *A. vera* contains vital components that have found applications in various treatments. GC-MS analysis of the extracts revealed the presence of some active constituents in the plant whose therapeutic potentials are discussed in this section.

4.1 Therapeutic potential and mechanisms of action

4.1.1 β-sitosterol

β-sitosterol, the most abundant identified compound (14.41%), is a plant sterol with cholesterol-lowering, anticancer, and immunomodulatory properties (Bin Sayeed and Ameen, 2015). It lowers cholesterol absorption in the gut and regulates the activity of genes related to cholesterol (Valerio and Awad, 2011) (Figure 3). β-sitosterol has demonstrated anticancer activity by inducing apoptosis and inhibiting angiogenesis (Yoon *et al*., 2018). Its immunomodulatory effects include enhancing natural killer cell activity and modulating cytokine production (Zhao *et al*., 2013). The cholesterol-lowering effect of β-sitosterol is primarily due to its structural similarity to cholesterol, which allows it to compete for incorporation into mixed micelles in the intestinal lumen. This competition decreases the intake of cholesterol from food and bile (Kim and Karadeniz, 2012). Furthermore, β-sitosterol has been demonstrated to regulate the activation of important genes related to cholesterol balance, such as Niemann-Pick C1-Like 1 (NPC1L1) and ATP-binding cassette transporters G5 and G8 (ABCG5/G8) (Racette *et al*., 2010). Various *in vitro* and *in vivo* studies have shown the anti-cancer effects of β-sitosterol. It has been demonstrated to trigger cell death in cancer cells through various methods, such as activating the Fas signaling pathway and the mitochondrial-dependent pathway. Furthermore, β-sitosterol hinders the growth of cancer cells by halting the cell cycle at the G2/M phase. Furthermore, it has also demonstrated anti-angiogenic properties by downregulating vascular endothelial growth factor (VEGF) expression and inhibiting endothelial cell tube formation. β-sitosterol has immunomodulatory effects that are characterized by its capacity to augment T-lymphocyte and natural killer cells. Research has shown that it enhances the production of Th1 cytokines (such as IL-2 and IFN-γ) and reduces the production of Th2 cytokines (such as IL-4). This modification of the immune system's reaction could help with its ability to fight cancer and reduce inflammation.

4.1.2 2-Furancarboxaldehyde, 5-(hydroxymethyl)-(HMF)

HMF (5.87%) is a furanic compound with antioxidant, neuroprotective, and anti-inflammatory properties (Saini and Keum, 2018). It has exhibited free radical scavenging activity and can protect neurons against oxidative stress-induced damage (Kaithwas *et al*., 2012). It has been demonstrated to block the generation of pro-inflammatory cytokines by inhibiting NF-κB and MAPK signaling pathways (Boudreau and Beland, 2006). HMF's antioxidant capability comes from its ability to neutralize different reactive oxygen species (ROS) like superoxide and hydroxyl radicals. This property contributes to its overall protective effects against oxidative stress-induced cellular damage (Kunle *et al*., 2012). HMF has been demonstrated to increase the levels of antioxidant enzymes like superoxide dismutase (SOD) and catalase in neuronal cells, thus boosting its ability to protect the brain (World Health Organization, 2003). The neuroprotective effects of HMF extend beyond its antioxidant properties. Studies have demonstrated that HMF can reduce β-amyloid aggregation and tau hyperphosphorylation, two

key pathological features of Alzheimer's disease. These effects, combined with its ability to improve cognitive function in animal models, suggest that HMF may have potential in the prevention or treatment of neurodegenerative disorders. The anti-inflammatory activities of HMF are mediated through its capability to modulate key inflammatory signaling pathways. HMF has demonstrated the ability to inhibit the activation of NF-κB, a transcription factor that is crucial in the process of inflammation. This suppression results in a reduction in the synthesis of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Furthermore, HMF inhibits the activation of MAPKs, adding to its anti-inflammatory properties.

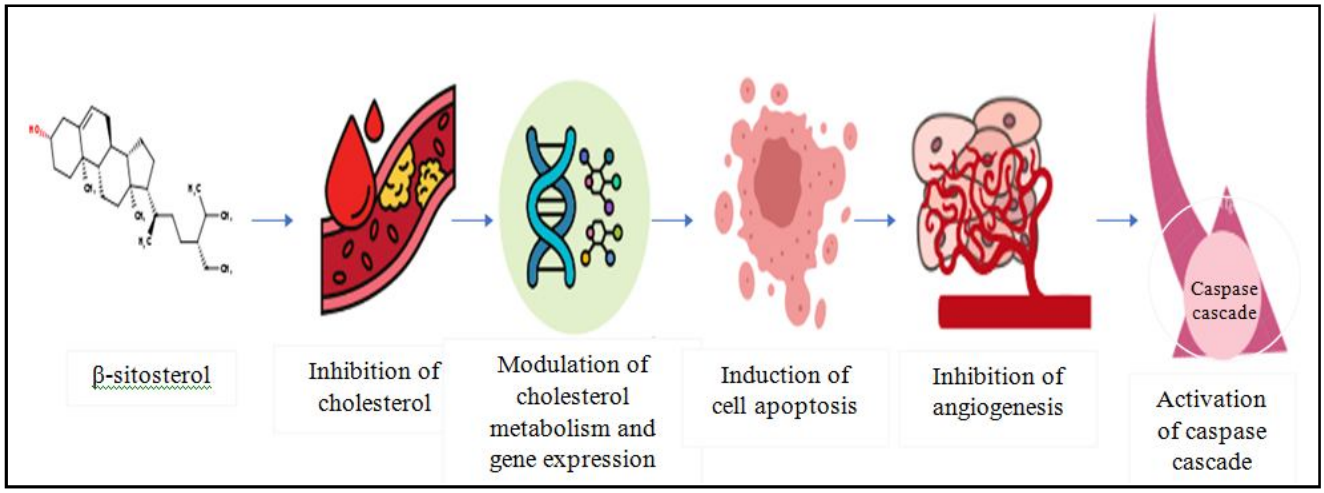

Figure 3: Proposed mechanism of action of β-sitosterol.

4.1.3 Lupeol

Lupeol (5.39%) is a pentacyclic triterpenoid with anti-inflammatory, anticancer, and antimicrobial properties (Woyengo *et al*., 2009). It prevents the generation of pro-inflammatory mediators by inhibiting NF-κB activation and MAPK signaling pathways (Figure 4). Lupeol initiates apoptosis in various cancer-causing cell lines and prevents tumor angiogenesis and metastasis (Hêœ *et al*., 2019). It also demonstrates antibacterial effects against Gram-positive bacteria and Gram-negative bacteria (Radha and Laxmipriya, 2015). Lupeol reduces inflammation by inhibiting the activation of NF-κB. Lupeol suppresses the expression of pro-inflammatory genes by blocking NF-κB translocation to the nucleus, which includes encoding cytokines like TNF-α, IL-1β, IL-6, and enzymes related to inflammation such as cyclooxygenase-2 and inducible nitric oxide synthase (Hamman, 2008). Lupeol also influences the function of MAP kinases, which helps enhance its anti-inflammatory effects (Surjushe *et al*., 2008). In cancer cells, lupeol has been demonstrated to initiate cell apoptosis through both the intrinsic (mitochondrial) and extrinsic (death receptor) pathways. It increases the expression of pro-apoptotic proteins like Bax and decreases the expression of antiapoptotic proteins such as Bcl-2. Lupeol also activates caspases, key enzymes in the execution of apoptosis. Additionally, lupeol inhibits cancer cell invasion and metastasis by modulating matrix metalloproteinases (MMPs) and reducing the expression of VEGF, a crucial factor in tumor angiogenesis. Lupeol has been shown to have antimicrobial effects against different pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The mechanism of action is thought to involve disruption of bacterial cell membranes, leading to cell lysis. Lupeol has also shown antifungal and antiparasitic properties, broadening its potential as an antimicrobial agent.

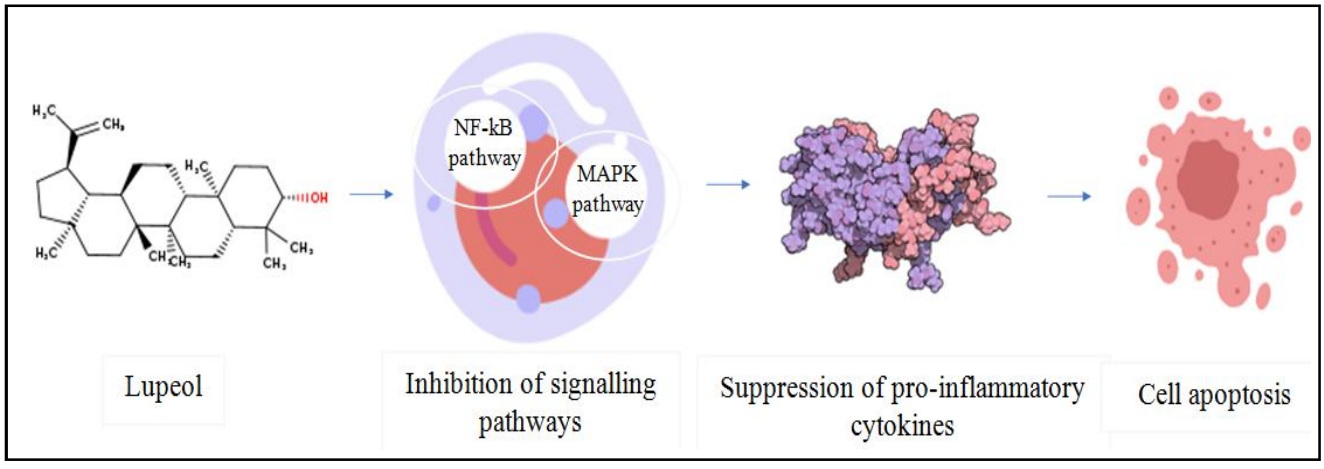

Figure 4: Proposed mechanism of action of lupeol.

4.1.4 Squalene

Squalene (2.93%) is a triterpene with antioxidant, anticancer, and immune-enhancing properties. It is a potent scavenger of singlet oxygen species and protects against lipid peroxidation. Squalene has shown chemo preventive effects in various animal models of cancer and is used as an adjuvant in some vaccine formulations due to its ability to enhance immune responses. Squalene's antioxidant properties mainly come from its capability to eliminate singlet oxygen, which is a very reactive form of oxygen that can lead to considerable oxidative damage to cellular components. Its antioxidant capacity is particularly important in protecting skin lipids from peroxidation, contributing to its use in dermatological and cosmetic applications. In terms of its anticancer properties, squalene has demonstrated chemo-preventive effects in various animal models of skin, colon, and lung cancer. One proposed mechanism is the prevention/ suppression of HMG-CoA reductase, an important enzyme in cholesterol biosynthesis, which may lead to a decrease in cell proliferation. Studies have also demonstrated that squalene can enhance the immune system's ability to detect and eliminate cancer cells. The immunity-boosting properties of squalene have led to its use as an adjuvant in vaccine formulations. In this scenario, squalene creates an oil-in-water emulsion that boosts the immune response to the vaccine antigen. It boosts the recruitment and activation of cells that present antigens, resulting in enhanced production of antibodies and responses from T-cells.

4.1.5 n-Hexadecanoic acid (Palmitic acid)

Palmitic acid (2.33%) has antimicrobial and anti-inflammatory properties. It has shown antibacterial activity against various pathogenic bacteria and can modulate inflammatory responses. However, its effects are complex and can be pro-inflammatory in some contexts, particularly in metabolic disorders. The antimicrobial activity of palmitic acid is thought to be due to its ability to disrupt bacterial cell membranes. Its hydrophobic nature allows it to interact with and potentially destabilize the lipid bilayer of bacterial cells, leading to cell apoptosis. This activity has been observed against Gram-positive as well as Gram-negative bacteria, including some antibiotic-resistant strains. The function of palmitic acid in inflammation is intricate and varies depending on the situation. Some research has demonstrated anti-inflammatory effects by inhibiting the generation of pro-inflammatory cytokines. Yet, in relation to metabolic issues like obesity and type 2 diabetes, heightened levels of palmitic acid have been associated with greater inflammation. This inflammatory response is believed to be aided by the activation of Toll-like receptor 4 (TLR4) signaling and the promotion of endoplasmic reticulum stress.

4.2 Safety and toxicological aspects

A. vera gel is generally regarded as safe for both oral consumption and topical application, but several important safety considerations warrant attention. The latex of *A. vera* contains anthraquinones, particularly aloin, which can act as a potent laxative. Prolonged use or high doses of aloin-containing products may lead to serious health issues such as electrolyte imbalances, kidney dysfunction, and hepatotoxicity. Furthermore, certain *A. vera* constituents have the potential to interact with medications, as exemplified by its hypoglycaemic effects potentially enhancing the action of anti-diabetic drugs. Although, rare, allergic reactions to *A. vera* have been documented, ranging from mild skin irritation to severe anaphylaxis in sensitive individuals. Some studies have reported cytotoxic effects of *A. vera* extracts at high concentrations *in vitro*, though these effects are generally not observed at physiologically relevant doses. Given these concerns, there is a pressing need for comprehensive toxicological studies to establish a thorough safety profile for *A. vera* extracts and their constituents. This is particularly crucial for assessing long-term use and potential effects on specific populations such as pregnant women and children. While, *A. vera* offers numerous potential benefits, these safety considerations underscore the importance of careful use and further research to fully understand its effects on human health.

4.3 Regulatory considerations

The development of standardized *A. vera* products necessitates strict adherence to quality control and regulatory guidelines, encompassing several key areas. Standardization is crucial, requiring the establishment of consistent methods for the cultivation, harvesting, processing, and extraction of *A. vera* to ensure uniform quality and potency across products. Rigorous quality control measures are essential, involving thorough testing for contaminants such as heavy metals, pesticides, and microorganisms, as well as standardization of bioactive compound content. Adherence to good manufacturing practices (GMP) guidelines is imperative to guarantee product quality, safety, and efficacy throughout the production process. Accurate labeling of *A. vera* products is vital, with any health claims requiring substantiation through scientific evidence. This ensures consumers are well-informed and protected from misleading information. Furthermore, there is a growing need for international harmonization of regulations to facilitate global trade and establish consistent standards for *A. vera* products across different countries. These regulatory considerations collectively aim to ensure that *A. vera* products meet high standards of quality, safety, and efficacy, thereby protecting consumers and maintaining the integrity of the industry. As the popularity of *A. vera* products continues to grow, these regulatory aspects will play an increasingly important role in shaping the market and ensuring public trust in these natural products.

4.4 Potential as adjuvant therapy

A. vera bioactive compounds have demonstrated significant potential as adjuvant therapies for a wide range of health conditions, offering promising complementary approaches to conventional treatments. In cancer therapy, compounds such as β-sitosterol and lupeol have shown synergistic effects with chemotherapeutic agents, potentially enhancing their efficacy while simultaneously reducing side effects. This dual action could lead to more effective and tolerable cancer treatments. For wound healing, *A. vera* gel components, particularly glucomannan and, gibberellin, may accelerate the healing process when used in conjunction with standard wound care treatments, potentially leading to faster recovery and reduced scarring. In the management of diabetes, *A. vera* extracts have exhibited the ability to improve glycaemic control when used alongside conventional antidiabetic medications, offering a complementary approach to managing this chronic condition. Furthermore, in dermatology, Aloe-based formulations show promise in enhancing the efficacy of topical treatments for various skin conditions, including psoriasis and atopic dermatitis. These formulations may help to reduce inflammation, soothe irritation, and improve overall skin health when used in combination with standard dermatological therapies. The potential

of *A. vera* as an adjuvant therapy across these diverse areas highlights its versatility and the growing interest in integrating natural products into mainstream medical treatments. However, it is important to note that while these findings are promising, further research and clinical trials are necessary to fully establish the efficacy and safety of *A. vera* compounds in these adjuvant roles.

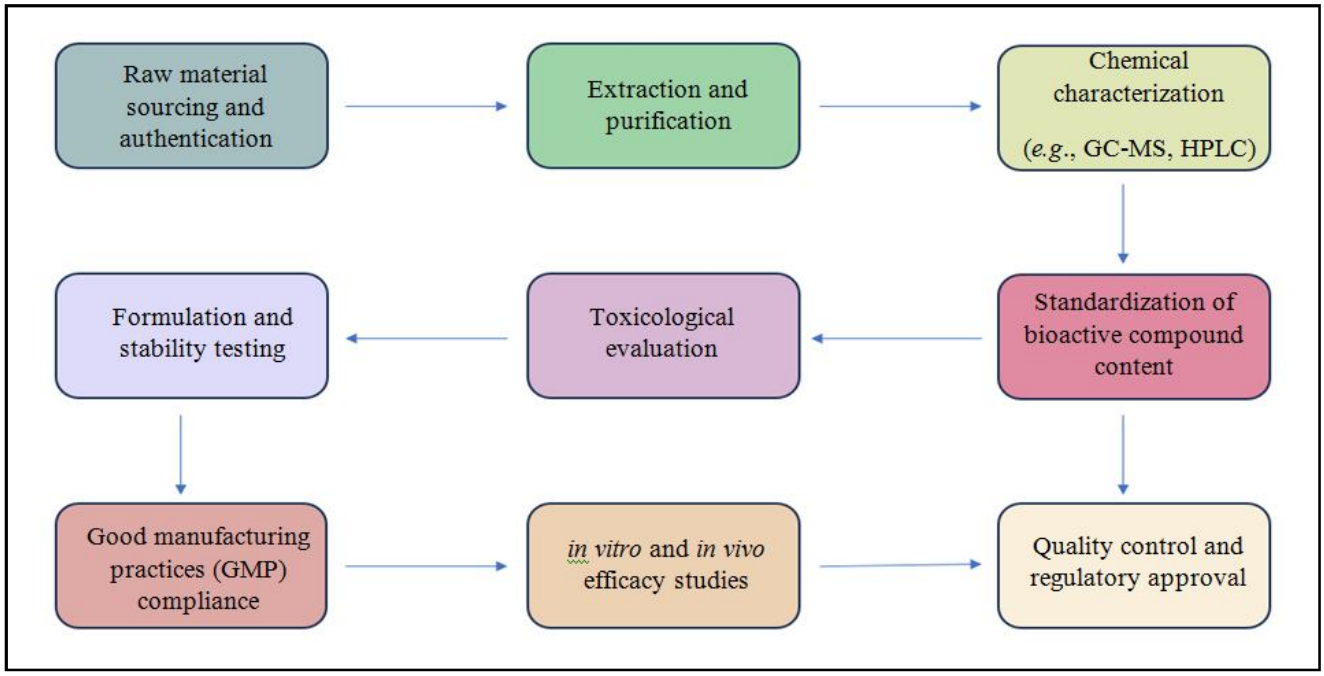

Figure 5: Schematic representation of the standardization and quality control process for *A. vera* products.

4.4.1 Strategies for improving the bioavailability and targeted delivery of *A. vera* bioactive compounds

Researchers are exploring various innovative strategies to enhance the bioavailability and targeted delivery of *A. vera* bioactive compounds, aiming to maximize their therapeutic potential (Figure 6). One promising approach involves the development of nanoparticle-based delivery systems, which can significantly improve the stability and bioavailability of *A. vera* compounds. These nanoformulations offer enhanced administration techniques that not only increase safety but also boost efficacy, potentially leading to improved treatment outcomes (Kaustubh Jadhav and Nupur Mehrotra (2022); Atharva Yatin Naik and Priya Sundarrajan (2023). Another strategy is liposomal encapsulation, where *A. vera* extracts or specific compounds are enclosed within liposomes, facilitating better absorption and cellular uptake. This method can protect the bioactive compounds from degradation and enhance their delivery to target tissues. Phytosome technology represents another advanced approach, involving the creation of complexes between *A. vera* compounds and phospholipids. This technique can markedly improve the absorption and tissue distribution of the bioactive compounds, potentially enhancing their therapeutic effects. Additionally, researchers are working on controlled-release formulations designed to provide sustained release of *A. vera* bioactive compounds, enabling prolonged therapeutic effects and potentially reducing dosing frequency. These diverse strategies collectively aim to overcome the limitations of traditional delivery methods, such as poor absorption or rapid metabolism, and to optimize the therapeutic potential of *A. vera* compounds. By improving bioavailability and targeting specific tissues or cells, these advanced delivery systems could significantly enhance the efficacy of *A. vera* -based treatments across various health applications. However, it's important to note that while these strategies show great promise, further research and clinical trials are necessary to fully validate their effectiveness and safety in human applications.

4.4.2 Future directions

The future of *A. vera* research and development is multifaceted, encompassing a wide range of scientific and practical endeavors. A primary focus is on conducting well-designed clinical trials to rigorously validate the therapeutic effects of *A. vera* extracts and compounds, providing solid evidence for their efficacy. Detailed pharmacokinetic and pharmacodynamic studies of *A. vera* bioactive compounds are crucial to understand their behaviour in the body and optimizing dosing regimens. Investigating potential synergistic effects between *A. vera* compounds and conventional medications could unlock new therapeutic strategies and improve existing treatments. The development and optimization of novel formulations to enhance bioavailability and targeted delivery remain key areas of research, potentially leading to more effective *A. vera* -based products. Long-term safety studies on prolonged use of *A. vera* products are essential to ensure their safety for extended use. Environmental considerations are also important, with efforts focused on exploring sustainable and eco-friendly methods for *A. vera* cultivation and processing. Advanced techniques like genomics and proteomics are being employed to elucidate the molecular pathways of *A. vera* compounds, deepening our understanding of their mechanisms of action. Researchers are also investigating *A. vera* potential in emerging therapeutic areas, such as neurodegenerative disorders, opening new avenues for its application. The development of standardized analytical methods

for quantifying bioactive compounds in *A. vera* products is crucial for ensuring product quality and consistency. Additionally, exploring *A. vera* potential in veterinary medicine could expand its therapeutic reach beyond human applications. Studies on the interactions between *A. vera* compounds and the gut microbiome may reveal new insights into its health benefits. Lastly, investigating the effects of different processing methods on *A. vera* bioactive compound profile is important for optimizing product formulation and preserving its beneficial properties. These diverse research directions collectively aim to enhance our understanding of *A. vera*, improve its therapeutic applications, and ensure its safe and effective use in various health contexts.

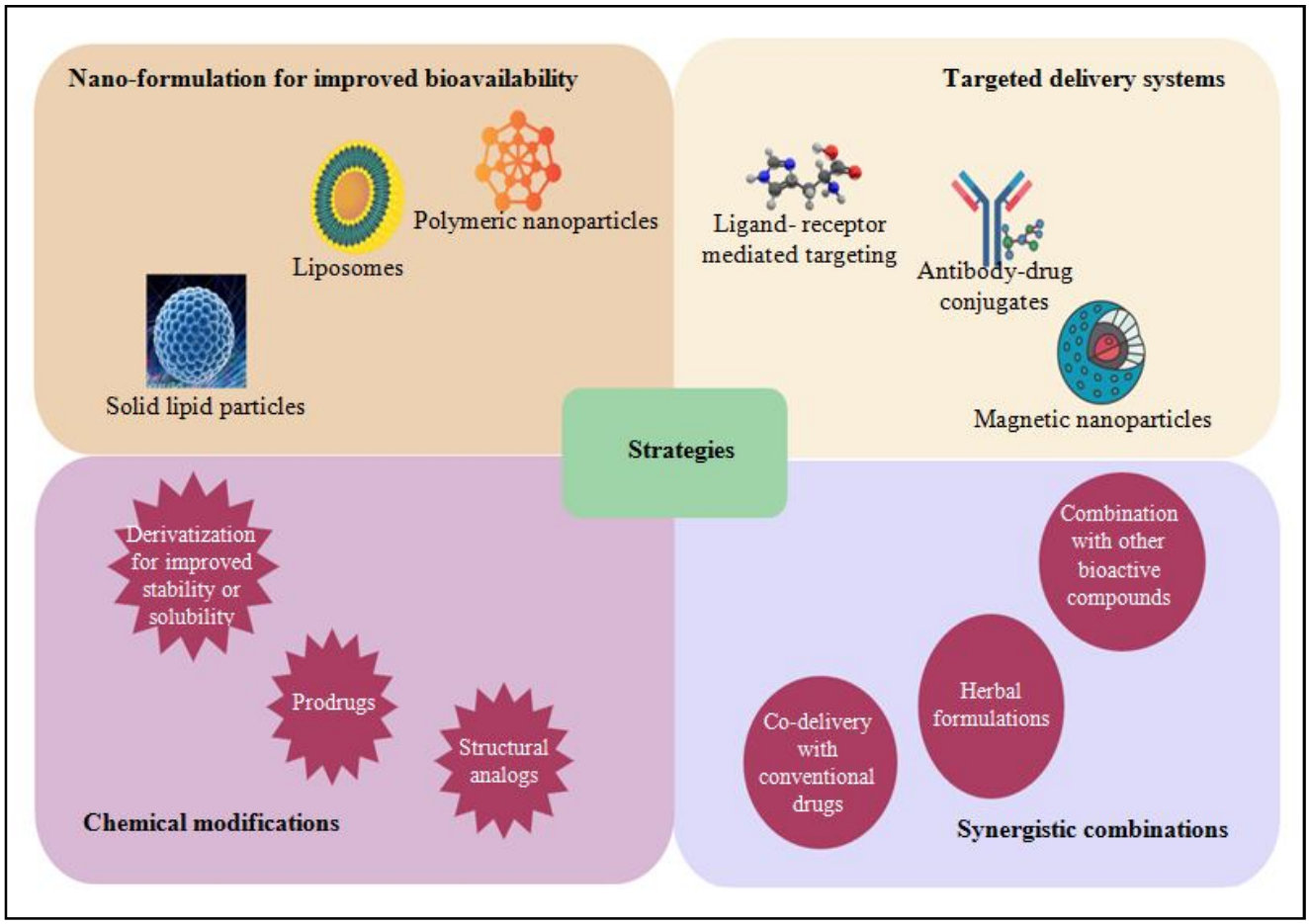

Figure 6: Potential strategies for enhancing the therapeutic efficacy of *A. vera* bioactive compounds.

5. Conclusion

This gas chromatography-mass spectroscopy (GC-MS) analysis of methanolic extracts from *A. vera* identified β-sitosterol, n-hexadecanoic acid, 2-furancarboxaldehyde, 5-(hydroxymethyl)-, and squalene as major bioactive compounds. These findings provide a scientific basis for *A. vera* traditional uses and reveal its diverse therapeutic potential, such as anti-inflammatory, anticancer, antimicrobial, and antioxidant properties. The study highlights the importance of applying modern analytical techniques to traditional medicinal plants, bridging ethnobotanical knowledge and evidence-based medicine. However, developing standardized, safe, and effective *A. vera* products requires addressing challenges in standardization, bioavailability, regulatory compliance, and sustainable sourcing. Interdisciplinary collaboration is crucial for realizing *A. vera*'s full potential sustainably and ethically. While this study provides a solid foundation, further research, including well-designed clinical trials, is necessary to fully validate the efficacy and safety of *A. vera* -based treatments. This research represents a significant step in the scientific exploration of *A. vera*, demonstrating both its immense potential and the challenges in translating traditional knowledge into modern therapeutic applications. With continued research, innovation, and collaboration, *A. vera* has the potential to make substantial contributions to human health, exemplifying the value of integrating traditional wisdom with modern science in addressing contemporary health challenges.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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