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REVIEW ARTCILE

Therapeutic Applications of Garlic (*Allium sativum* L.) Essential Oil: A Review on Different Extraction Methods and Characterization on Its Bioactive Compound

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Therapeutic Applications of Garlic (Allium sativum L.) Essential Oil: A Review on Different Extraction Methods and Characterization on Its Bioactive Compound

SUMMARY

Garlic (Allium sativum L.) has long been extensively recognized for its significant role as a culinary ingredient and traditional medicine, primarily attributed to its essential oil, which is rich in bioactive compounds with potential therapeutic effects. This review explores the therapeutic applications of Allium sativum essential oil (ASEO), with particular attention to the influence of various extraction techniques on the chemical composition of ASEO. The techniques include hydrodistillation, steam distillation, simultaneous distillation-extraction (SDE), microwave-assisted hydrodistillation (MAHD), expanded solvent extraction (ESE), Supercritical fluid carbon dioxide (SCF-CO_2) extraction, and Soxhlet extraction. The majority of literature has reported that diallyl sulfides (DAS) and their derivatives consistently emerge as the predominant bioactive constituents, contributing to the oil's antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer activities. Furthermore, this review addresses concerns regarding the potential toxicity of specific compounds present in ASEO and highlights challenges associated with its limited bioavailability and physicochemical stability. Future research should prioritize the development of green extraction methods and the implementation of encapsulation technology on ASEO for enhancing the therapeutic efficacy and applicability of ASEO.

Keywords: *ASEO, extraction method, diallyl sulfides, therapeutic application.*

Sarımsak (Allium sativum L.) Uçucu Yağının Terapötik Uygulamaları: Farklı Ekstraksiyon Yöntemleri ve Biyoaktif Bileşiğinin Karakterizasyonu Üzerine Bir Derleme

ÖZ

Sarımsak (Allium sativum L.), potansiyel terapötik etkileri olan biyoaktif bileşikler açısından zengin olan uçucu yağı sayesinde, uzun zamandır mutfak malzemesi ve geleneksel tıpta önemli bir rol oynadığı bilinen bir bitkidir. Bu derleme, Allium sativum uçucu yağının (ASEO) terapötik uygulamalarını, çeşitli ekstraksiyon tekniklerinin ASEO'nun kimyasal bileşimi üzerindeki etkisine dikkat çekerek incelemektedir. Bu teknikler arasında hidrodistilasyon, buhar distilasyonu, eşzamanlı distilasyon-ekstraksiyon (SDE), mikrodalga destekli hidrodistilasyon (MAHD), genişletilmiş çözücü ekstraksiyonu (ESE), süperkritik sıvı karbondioksit (SCF-CO_2) ekstraksiyonu ve Soxhlet ekstraksiyonu bulunmaktadır. Literatürün büyük bir kısmında, dialil sülfürler (DAS) ve türevlerinin, yağın antibakteriyel, antifungal, antiviral, antioksidan, antiinflamatuar ve antikanser aktivitelerine katkıda bulunan başlıca biyoaktif bileşenler olarak ortaya çıktığı bildirilmiştir. Ayrıca, bu derleme ASEO'da bulunan belirli bileşiklerin potansiyel toksisitesine ilişkin endişeleri ele almakta ve sınırlı biyoyararlanımı ve fizikokimyasal stabilitesi ile ilişkili zorlukları vurgulamaktadır. Gelecekteki araştırmalar, ASEO'nun terapötik etkinliğini ve uygulanabilirliğini artırmak için yeşil ekstraksiyon yöntemlerinin geliştirilmesine ve ASEO'da kapsülleme teknolojisinin uygulanmasına öncelik vermelidir.

Anahtar Kelimeler: *ASEO, ekstraksiyon yöntemi, dialil sülfürler, terapötik uygulama.*

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INTRODUCTION

The genus *Allium*, which is the largest within the family Alliaceae, encompasses approximately 700 identified species. The most widely consumed members include *Allium cepa* L. (onion), *A. sativum* L. (garlic), *A. porrum* L. (leek), *A. schoenoprasum* L. (chives), *A. fistulosum* L. (Welsh onion), and *A. ascalonicum* L. (shallots). The species under this genus have historically been utilized as staple vegetables and ingredients in culinary components across a broad spectrum of global dietary traditions (Mai et al., 2020; Upadhyay, 2017). The *Allium* genus, whose origins are primarily in Central Asia, has undergone extensive cultivation due to its unique organoleptic properties, adaptability to diverse growing conditions, and favorable post-harvest longevity (Benkeblia & Lanzotti, 2007). *Allium sativum* L., more generally known as garlic, is an herbaceous plant that has been used for millennia in numerous cultures worldwide (Corzo-Martínez et al., 2007). A distinguishing characteristic of garlic is the presence of essential oils, which play a significant role in its functional and therapeutic properties (Corzo-Martínez et al., 2007).

The essential oil obtained from *Allium sativum* L. (ASEO) exhibits strong antimicrobial and antioxidant activities that are largely attributed to its volatile organosulfur compounds such as allicin, diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene, and related polysulfides. These compounds produce antimicrobial effects by reacting with thiol (–SH) groups of bacterial enzymes and low-molecular-weight thiols, thereby inhibiting essential metabolic enzymes, and by disrupting bacterial membrane integrity, which leads to leakage of cellular contents and loss of membrane potential (Bhatwalkar et al., 2021). Mnayer et al. (2014) found that these compounds effectively treat various diseases (Mnayer et al., 2014). Several preclinical studies have reported that ASEO can support the balance of the immune system, particularly in the gastrointestinal tract (GIT), by enhancing anti-inflammatory activity and reducing pro-inflammatory mediators (Subudhi et al., 2024). Various studies have demonstrated that key constituents such as allicin, diallyl disulfide (DADS), and diallyl trisulfide (DATS) possess a wide range of biological functions, including antibacterial, antiviral, antifungal, antioxidant, and anti-inflammatory properties. Diverse mechanisms, including the suppression of inflammatory signaling pathways, regulation of oxidative stress, and direct antimicrobial actions, mediate these effects (Dehariya et al., 2021). Despite the established benefits of ASEO, a more thorough examination of its chemical composition and characteristics is necessary. Hydrodistillation is the conventional method for extracting essential oils. However, recent studies have introduced alternative extraction techniques, and the method of extraction significantly influences the chemical profile and subsequent bioactivity of ASEO. The challenges associated with the application of ASEO are stability and bioavailability. Encapsulation techniques may help to enhance these properties, thereby improving the therapeutic efficacy (Bhatwalkar et al., 2021).

This review article discusses various methodologies for extracting bioactive compounds from garlic oil and their subsequent impact on the physical, chemical, and biological properties of the oil. The article also discusses the toxicity of ASEO and its potential applications in the pharmaceutical and nutraceutical industries. This review also points out the importance of ASEO in the development of novel pharmaceutical and nutraceutical products.

MATERIALS AND METHODS

The methodology for this review involved a comprehensive analysis of studies on the extraction, characterization, and therapeutic applications of garlic (*Allium sativum* L.) essential oil (ASEO). Relevant literature was sourced from reputable international scientific databases, including Springer, Elsevier, Google Scholar, and PubMed, prioritizing peer-reviewed articles from high-impact journals to ensure validity and relevance. Some queries and specific keywords were generated to highlight the bioactive compounds and pharmacological properties of ASEO, focusing on the therapeutic properties of ASEO.

Extraction method and characterization of *A. sativum* L. essential oil (ASEO)

The selection of the extraction method is important when extracting a chemical compound. A variety of methods can be employed to extract essential oils from the *A. sativum* L. plant. One method that is both widespread and commonly used is hydrodistillation. Garlic hydrodistillation using Clevenger for a period of three hours can yield pale yellow ASEO with varying yields of 0.2%, 0.22%, and 0.18%, according to Satyal et al. (2017). ASEO can be separated by decanting and adding sodium chloride, then stored in the refrigerator at -4°C. In other studies conducted by Douiri et al. (2013), using the hydrodistillation method, the yield was 0.32% ± 0.2 of the weight of fresh garlic.

Another method that can be used in addition to hydrodistillation is the soxhlet extraction technique.) According to Dehariya, Guha, and Gupta, (2021), the optimization of ASEO extraction treatment yielded optimal experimental conditions, with a temperature of 50°C and a duration of 4 hours, resulting in a maximum extraction yield of 16.55% (db). The method employed exerts a discernible influence on the ASEO obtained, manifesting in variations both in terms of yield and quality. Conventional extraction methods such as hydrodistillation and soxhletation are still widely employed due to their ease of operation. However, current research has revealed the emergence of numerous advanced extraction methodologies.

Supercritical fluid carbon dioxide (SCF-CO_2) extraction has been reported as a means to extract ASEO, yielding a 5% yield. Essential oils obtained by this method possess the distinctive flavor of *A. sativum* L., while the steam method yields oils with an identifiable garlic aroma. The fresh taste of *A. sativum* L. is possible due to both saturated and unsaturated thiosulfinates and thiosulfonates in the extract obtained by the supercritical fluid carbon dioxide (SCF-CO_2) method (Mabe & Bottini, 2014). In contrast, the hydrodistillation method, which utilizes elevated operating temperatures, has been observed to result in the loss of these compounds. Essential oils obtained through supercritical fluid carbon dioxide (SCF-CO_2) extraction mostly contain methyl and methyl-allyl polysulfide compounds. SCF-CO_2 extraction has been reported to produce essential oil yields of approximately 7% and 6%, surpassing the 5.5% yield typically obtained through conventional steam distillation. Several physical parameters, particularly the particle size and morphology of the plant material during extraction, play a critical role in determining yield and extraction efficiency. Particle agglomeration, which often results from grinding, can hinder extraction performance by reducing the surface area available for solvent interaction and lengthening diffusion pathways within the solid matrix, thereby restricting effective mass transfer. Therefore, variations in parameters such as particle size, extraction temperature, pressure, and duration can significantly impact the yield and composition of the essential oil obtained from *A. sativum* L. (Rafe & Nadjafi, 2017).

Microwave-assisted extraction (MAE) has many advantages, including a short extraction process and a high yield. The magnetic radiation emitted by the solvent-matrix system, rapidly increasing the temperature. Compared to conventional methods, which generally take about 30 minutes, the extraction process subjects the sample to significant thermal stress. Another benefit of this extraction that it is performed at under room temperature conditions, in contrast to conventional distillation methods that require elevated temperatures (up to 100°C). This characteristic enables the process to minimize or potentially avoid thermal degradation associated with heat-intensive treatments commonly applied during traditional distillation (Dziri et al., 2014). For example, MAE has been shown to produce essential oil containing 17 major compounds and exhibiting strong antioxidant and lipid peroxidation inhibitory activities, including DPPH scavenging of 72.06% and nitric oxide inhibition of 79.96% (Bajpai et al., 2015). Comparative analyses of extraction techniques reveal significant differences in yield, purity, and biological activity of ASEO. Hydrodistillation, while widely used, often leads to thermal degradation of key sulfur compounds, including allicin and diallyl trisulfide (DATS). In contrast, methods like supercritical CO_2 extraction and microwave-assisted hydrodistillation (MAHD) offer greater efficiency in preserving these thermolabile compounds, leading to enhanced antibacterial, antifungal, and anticancer activities. Supercritical CO_2 extraction, in particular, enables the isolation of unique unsaturated thiosulfinates with minimal oxidation, enhancing the functional properties of ASEO. This suggests that advanced extraction techniques not only increase yield but also preserve the therapeutic quality of the essential oil (Bajpai et al., 2015; Okoh et al., 2010).

***A. sativum* L. essential oil (ASEO) chemical profile**

The chemical profile of ASEO is very complex. Garlic itself comprises approximately 2,000 biologically active constituents, including a diverse array of organosulfur compounds that may be unstable, water-soluble, or oil-soluble in nature (Bazaraliyeva et al., 2022). Consequently, disparities in processing methodologies can exert a substantial impact on the ultimate composition of garlic preparations. In the array of available forms, the raw garlic homogenate has the highest degree of consumption and is characterized by its substantial allicin content. However, due to its inherent instability, allicin readily undergoes conversion into other sulfur-containing derivatives (Nakamoto et al., 2019).

The majority of the extraction techniques that have been employed to date have been designed to isolate these sulfur-based compounds, which are recognized as the principal contributors to garlic's pharmacological activity. Comprehensive evaluations of garlic's bioactive constituents have consistently indicated that sulfur-containing compounds possess a broad spectrum of therapeutic effects (Bazaraliyeva et al., 2022). Some formulations are designed to release allicin reliably and may feature enteric coatings or other delivery technologies. These different product types reflect the diversity of garlic's bioactive compounds, such as allicin, diallyl disulfide, and S-allyl cysteine, and the fact that their stability, bioavailability, and physiological effects vary substantially (Verma et al., 2023). Currently, various commercial garlic products are available, including garlic oil, garlic powder, and standardized extracts such as aged garlic extract (AGE), which are formulated to enhance the stability and bioavailability of sulfur compounds (Sasi et al., 2021). Modern formulations such as nanoemulsions and encapsulated garlic essential oils have been developed to improve solubility, controlled release, and therapeutic efficacy (Hassanzadeh et al., 2022; Liu et al., 2018). Standardized products like AGE are often characterized by consistent levels of S-allyl-cysteine, ensuring reproducible pharmacological effects across batches (Valls et al., 2022). A comparative summary of ASEO compositions derived from various extraction approaches is presented in Table 1.

Table 1. Chemical composition of ASEO

No	Extraction method	Main component	Reference
1	Hydrodistillation	DAS (1.9–9.5%), DADS (20.8–27.9%), DATS (16.8–33.4%), allyl methyl disulfide (AMDS) (4.4–8.3%), and allyl methyl trisulfide (AMTS) (14.5–19.2%).	(Satyal et al., 2017)
		di-2-propenyl trisulfide (46.52%), di-2-propenyl disulfide (16.02%), methyl 2-di-2- propenyl trisulfide (10.88%), and DADS (7.15%).	(Douiri et al., 2013)
		DADS (22.08%), AMTS (9.72%), 2-vinyl-4H-1,3-dithiine (4.78%), and α-bisabolol (3.32%).	(Herrera-Calderon et al., 2021)
		DADS (29.2%), DATS (28.6%), and AMTS (19.8%).	(Alnomasy, 2021)
		DATS (37.3–45.9%), DADS (17.5–35.6%), AMTS (7.7–10.4%), and the 2-vinyl-1,3-dithiane (3.9–5.9%).	(Dziri et al., 2014)
2	Steam distillation	DATS (27.33%); DADS (24.67%); AMTS (19.11%); propenyl dithiopropanoate (8.59%); dimethyl trisulfide (DMTS) (2.18%); Diallyl tetrasulfide (DTS) (2.13%); 3-vinyl-[4H]-1,2-dithiin (1.49%), and 2-vinyl-[4H]-1,3-dithiin (1.25%).	(Rohani et al., 2011)
		DADS (48.42%), AMTS (7.27%), trisulfide, di-2 propenyl (3.46%) and DAS (7.64%).	(Dehariya et al., 2021)
		Di-2-propenyl trisulfide (32.82%) and di-2-propenyl disulfide (29.12%), methyl 2-propenyl trisulfide (7.4%), DTS (6.35%), 2-Vinyl-4H-1,3-dithiin (5.87%).	(Pyun & Shin, 2006)
3	Simultaneous distillation solvent extraction (SDE)	DADS (28.4%), DATS (20.4%), AMTS (16.3%), cis and trans AMDS (9.3%), DAS (2.3%), dimethyl disulfide (DMDS) (2.2%), dimethyl trisulfide (DMTS) (2.0%), DTS (0.7%), 1,4-dimethyl tetrasulfide (0.4%), and traces of ethyl vinyl sulfide.	(Kimbaris et al., 2006)
4	Microwave-assisted hydrodistillation extraction (MAHD)	DADS (17.6%), DATS (9.1%), cis and trans AMTS (14.1%), AMDS (9.1%), DAS (0, 9%), dimethyl disulfide (DMDS) (2.1%), DMTS (2.7%), DTS (0.1%), 1,4-dimethyl tetrasulfide (0.4%), and also traces of ethyl vinyl sulfide.	(Kimbaris et al., 2006)
5	Ultrasound-assisted extraction (USE)	2-vinyl-[4H]-1,3-dithiin (38.1%) and 3-vinyl-[4H]-1,2-dithiin (32.7%). DADS (8.2%), DATS (0.2%), AMTS (0.1%), cis and trans AMDS (0.6%), DAS (0.3%), DMDS, DMTS, DTS, and traces of 1,4-dimethyl tetrasulfide. Ethyl vinyl sulfide was found in a measurable percentage (3.2%).	(Kimbaris et al., 2006)
6	Supercritical fluid carbon dioxide (SCF-CO_2)	4-mercaptoethanol (72.14%), 5-isopropyl tetrahydrothiophene-3-one (3.19%), 2-Furanmethanol (2.46%), 2-Furancarboxaldehyde (2.37%).	(Mabe & Bottini, 2014)
7	Soxhlet extraction	Ajoene	(Dharshini & Devi, 2017)

As demonstrated in Table 1, different extraction methods will produce different essential oil components. Based on several methods used in several studies, this can be due to the enzymatic biotransformation process that occurs during the extraction process. For example, a comparison of hydrodistillation and microwave extraction methods reveals significant disparities in the resulting components (Kimbaris et al., 2006). Water-extraction methods effectively isolate key sulfur-containing compounds, including allicin, allyl sulfide, diallyl sulfide, and methanethiosulfonic acid S-methyl ester, while ajoene is mostly obtained through Soxhlet extraction.

In addition, the type of equipment used in the extraction process can significantly influence the yield and composition of the essential oil. For instance, essential oils obtained using a Clevenger apparatus may differ in chemical profile and concentration compared to those extracted on an industrial scale. Moreover, environmental and agronomic factors such as soil characteristics and planting altitude also affect the phytochemical composition of garlic. To date, most garlic collections have been limited to low-altitude areas ranging from 500 to 1,000 meters above sea level (Dziri et al., 2014).

The chemical composition of ASEO is strongly influenced by the presence and relative abundance of volatile organosulfur compounds, which account for its characteristic aroma and variability (Rohani et al., 2011). The concentration and composition of these sulfur-containing compounds can be significantly affected by extraction parameters such as temperature and duration. Organosulfur compounds, including DADS, allicin, methyl-1-propenyl disulfide, allyl methyl trisulfide (AMTS), and other minor constituents, have been extensively investigated for their diverse biological activities. These compounds exhibit potent antioxidant, immunomodulatory, anticarcinogenic, anti-inflammatory, antidiabetic, antimicrobial, hypoglycemic, antihypertensive, and anticholesterolemic properties, making them the key contributors to the therapeutic potential of garlic essential oil (Verma & Srivastava, 2020).

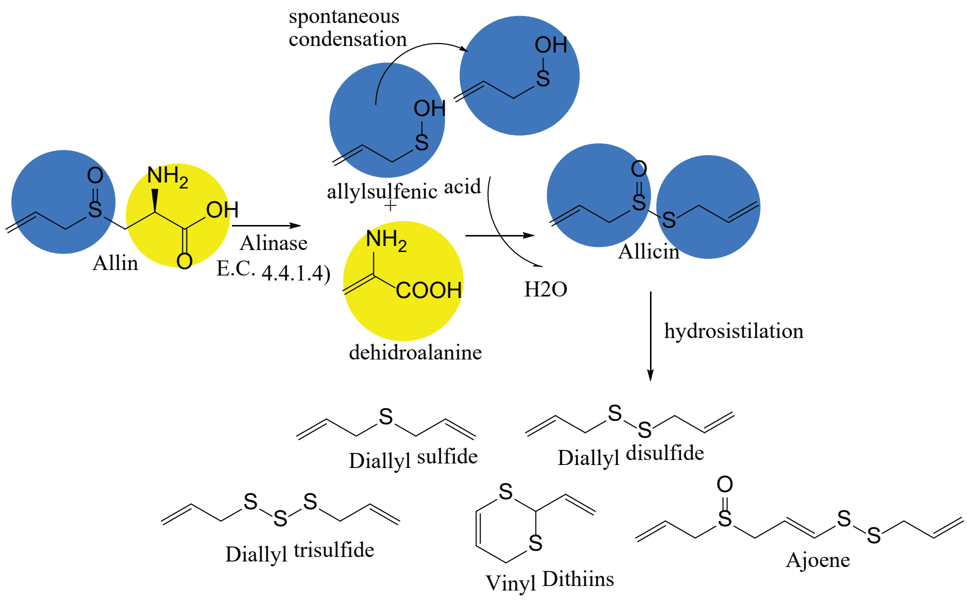

Figure 1. Conversion of Allin into several main components found in ASEO. Adapted from Borlinghaus et al., 2021.

Therapeutic action and bioactive compound mechanism of *Allium sativum* L. essential oil (ASEO)

ASEO contains compounds produced from thiosulfate during steam processing that are not present in the whole plant due to changes during the extraction and processing of the essential oil (Khoshtinat et al., 2016). This thiocyanate is a very unstable compound and undergoes further rearrangement reactions that produce various forms of sulfur compound derivatives through further transformations, and remains biologically active. The most representative substance among sulfonate compounds is the allyl compound, which is the most dominant compound in ASEO (Huang et al., 2023).

Sulfur-containing compounds derived from cysteine-based precursors have been identified as key contributors to the biocidal effects against phytophagous and pathogenic microorganisms (Douiri et al., 2013). While the precise antimicrobial mechanism remains to be elucidated, research suggests that this organosulfur compound functions by impeding the synthesis of DNA, RNA, and protein. This, in turn, prompts a reaction with the sulfhydryl groups of microbial enzymes and proteins, resulting in the degradation of cell walls and membranes and the compromise of cell membrane integrity (Alnomasy, 2021). The antimicrobial activity of allyl sulfur compounds (ASEs) is enhanced with each addition of S atoms. In addition, the antimicrobial activity of ASEs has been found to be stronger than that of individual compounds (Khoshtinat et al., 2016).

Figure 2. Schematic representation of the therapeutic actions of ASEO

Antibacterial activity

ASEO has excellent antibacterial activity. Garlic is rich in non-phytotoxic compounds and bioactive agents that enhance its effectiveness in microbial control (Hu et al., 2016; Prakash et al., 2015). Among these, the antibacterial compound DATS, commonly known as allicin, is notable for its spicy flavor, aroma, and potent antibacterial activity. Garlic's bioactive compound, allicin, is formed when the cloves are crushed. This reaction occurs between the amino acid alliin and the enzyme alliinase. The antimicrobial activity of ASEO is largely attributed to diallyl sulfide derivatives such as DAS, DADS, DATS, and DTS (Lawson et al., 1991).

The chemical composition and proportion of ASEO exhibit significant variation depending on the variety, geographic origin, harvest season, climate, and processing methods (Cui et al., 2016; Khan et al., 2010). These essential oils demonstrate a multifaceted mechanism of action against pathogenic bacteria, including membrane disruption, protein denaturation, enzyme interaction, ATP synthesis inhibition, induction of oxidative stress, and biofilm formation inhibition. The Gram-negative bacterial outer membrane, which is abundant in lipopolysaccharides, functions as a barrier, thereby protecting the cells from the hydrophobic compounds present in essential oils. This property contributes to the enhanced resistance exhibited by Gram-negative bacteria in comparison to Gram-positive bacteria (Torpol et al., 2018).

Nevertheless, ASEO has been demonstrated to exhibit antibacterial properties against various pathogenic bacteria, including *Pseudomonas*, *Proteus*, *Salmonella*, *S. aureus* (Cavallito & Bailey, 1944), *E. coli* (Johnson & Vaughn, 1969), *Klebsiella* (Benmeziane et al., 2018), *Clostridium botulinum* (Wit et al., 1979), *Mycobacterium* (Cavallito & Bailey, 1944), and *Helicobacter pylori* (O'Gara et al., 2000). The broad-spectrum antimicrobial activity of ASEO underscores its potential as a natural alternative to synthetic antimicrobial agents, warranting further investigation and application in various fields.

Antifungal activity

ASEO has been found to have antifungal properties, making it a crucial element in the treatment of various fungal infections that can affect different bodily regions, such as the skin, nails, hair, mouth, and genital organs. The antifungal properties of ASEO are primarily derived from allicin, a compound formed from DADS and DATS. The concentration of these sulfur-containing compounds varies depending on the geographic origin of the plant. According to Avato et al. (2000), DADS exhibits higher antifungal activity compared to DTS, with a general correlation observed between increased DADS content and enhanced inhibitory effects.

Interestingly, while DADS shows potent antifungal properties, it exhibits weaker antibacterial effects. Structurally, DADS and DATS differ in the number of sulfide bonds, with DADS containing fewer sulfide bonds than DADS, which may account for the variation in their biological activity.

Sun et al. (2017), demonstrated that DADS actively inhibits various pathogens, including *Fusarium oxysporum*, *Botrytis cinerea*, *Phytophthora capsici*, and *Verticillium dahliae*. Similarly, the study by Wang et al. (2019), reported that ASEO exhibited the capacity to impede the proliferation of *Candida* species, which are fungi that are responsible for infections in moist body areas, by causing damage to and leakage of the cytoplasmic membrane. Furthermore, Zhang et al. (2015), concluded that the mode of action of ASEO in inhibiting fungal growth involves compromising the permeability of the mycelial plasma membrane. The increase in cell membrane permeability induced by DADS leads to intracellular electrolyte leakage and ultimately cell death. These effects confirm that essential oils significantly disrupt the lipid layer of cell membranes, leading to intracellular leakage and subsequent fungal cell lysis.

Antiviral activity

The antiviral compounds found in ASEO include garlicin, allitridin, AMTS, vinyldithiins, (E/Z)-ajoene, and allicin. These compounds present in the essential oil are typically obtained through steam distillation and extraction methods Staba, Lash, and Staba (2018). Ma and Yao (2020), also reported that these compounds exhibit activity against various viruses, including influenza virus (IFV), human herpes virus (HSV), human immunodeficiency virus (HIV), and avian influenza. The antiviral mechanism of these essential oils involves the following: first, the oils adsorb the virus into host cells; second, they act as a blocker during the replication process. Furthermore, the organosulfur compounds present in *A. sativum* L. have exhibited antiviral properties by impeding viral attachment and penetration into host cells, as well as by suppressing viral enzymes such as RNA polymerase and reverse transcriptase. Table 2 explains several garlic-derived essential oil compounds with potential antiviral applications.

Table 2. Antiviral activity of ASEO

No	Extraction method	Essential oil compound	Virus	Reference
1	Steam-assisted co-distillation (SCD)	Garlic oil (50 μg/ml)	New Castle	(Hizam et al., 2019)
		Ajoene and Allicin	Herpes simplex virus	(Ushijima et al., 2018)
			COVID-19	(Alam et al., 2016; Asif et al., 2020)
2	GC-MS	DADS-28.4%, DATS-22.8%), allyl (E)-1-propenyl disulphide-8.2%, AMTS-6.7%, and DTS-6.5%	COVID-19	(Thuy et al., 2020)
3	Hydrodistillation	DATS-44.21% DADS-22.08% AMTS-9.72% 2-Vinyl-4H-1,3-dithiine-4.78% α-Bisabolol – 3.32%	IFV, SARS-CoV, MERS-CoV, SARS-CoV-2, Respiratory Syncytial Virus (RSV), Parainfluenza viruses (HPIV-1, HPIV-2, HPIV-3, HPIV-4), Human Rhinoviruses (HRVs), Metapneumovirus (hMPV), Hepatitis A virus (HAV), and Hepatitis E virus (HEV)	(Herrera-Calderon et al., 2021)

ASEO has antiviral effects through multiple mechanisms. One of the important mechanisms includes the suppression of angiotensin-converting enzyme 2 (ACE2), which serves as the primary receptor for SARS-CoV-2 to enter human cells. Garlic essential oil inhibits ACE2, preventing viral binding and subsequent infection. In addition, the organosulfur compounds present in ASEO have been shown to impede the function of the main protease (PDB6LU7) of SARS-CoV-2, thereby hindering viral replication (Qiu et al., 2020)

Furthermore, ASEO has been shown to possess significant antioxidant and immunomodulatory properties. This approach is predicated on the premise that oxidative stress and inflammation, both of which play important roles in viral pathogenesis, can be mitigated. Allicin, a notable compound present in garlic, has been demonstrated to S-thioallylate viral proteins, thereby modulating their function and constraining the replication of viruses at diverse stages of the infection cycle (Qiu et al., 2020).

A recent study shows that allicin can inhibit viral receptor binding, replication, or transcription by S-thioallylating host or viral proteins, effectively preventing viral entry into host cells (Jeong et al., 2016). S-thioallylation is a chemical process in which allicin interacts with sulfhydryl groups (-SH) on proteins to generate thioether linkages (-SS), affecting protein structure and function and so preventing SARS-CoV-2 infection (Mösbauer et al., 2021).

According to these findings, ASEO has promise as a supportive therapeutic or preventive approach against viral infections, particularly COVID-19. While further research is needed to properly understand its mechanisms, existing evidence suggests a promising role in antiviral treatment (Giordano et al., 2023).

Antioxidant activity

Oxidative stress is defined as a physiological imbalance between the generation of reactive oxygen species (ROS) and the efficacy of the body's antioxidant defence mechanisms to neutralize them. Studies have demonstrated the role of oxidative stress in the pathogenesis of chronic illnesses, such as cardiovascular disease and cancer (Tsai et al., 2012). Therefore, it is important to explore the potential of compounds that can act as antioxidants and prevent diseases caused by oxidative stress.

It has been demonstrated that ASEO exhibits notable antioxidant activity. However, it should be noted that the reported antioxidant activity can vary depending on the analysis method used. In addition, the extraction method has been demonstrated to influence antioxidant activity (Lawrence & Lawrence, 2011). In a study examining the antioxidant activity of ASEO from the garlic cultivated in the plains of northern India, the researchers concluded that the essential oil exhibited remarkable antioxidant properties, with an EC_{50} value of 500 µg/mL as determined by the DPPH free radical scavenging assay.

A comparative analysis of fresh *A. sativum* L. and its derivatives revealed that minced *A. sativum* L. exhibited significantly stronger antioxidant activity. This may be due to the extraction process, which enhances stability. Additionally, organosulfur compounds are active and soluble in water. Notable examples include S-allylcysteine and S-allylmercaptocysteine, both of which possess antioxidant activity. Some allyl compounds found in ASEO are DAS, DADS, DATS, and diallyl polysulfide. These compounds have been shown to have antioxidant properties based on DPPH and TEAC assays (Huang et al., 2023).

Herrera-Calderon et al. (2021), found that the antioxidant activity of ASEO obtained through hydrodistillation was more potent than that obtained through ultrasound-assisted or sonohydrodistillation, as evaluated using the DPPH assay. The IC_{50} value for hydrodistilled ASEO was 0.96 mg/mL. The values for the other two methods were 1.176 and 1.234 mg/mL, respectively. It is essential to acknowledge that various factors, such as altitude, climate, and chemotype variations, can impact these results. Another analysis conducted by Herrera-Calderon et al. (2021), demonstrated that ASEO obtained through hydro distillation exhibited an IC_{50} value of 6.17 µg/ml (42.93%), which was evaluated using the DPPH method. Conversely, Yasin et al. (2022), proposed that its antioxidant activity is based on the percentage of DPPH free radicals scavenged, which was reported to be 80% (0.24 mg quercetin per gram, and 0.33 mg gallic acid per gram).

Furthermore, the influence of temperature on the antioxidant activity of ASEO has been evidenced (Dehariya et al., 2021), an increase in temperature of 10°C is associated with increased antioxidant activity. This may be attributed to the increased activity of phenolic and sulfur compounds at 70°C in comparison to 50°C and 60°C or to the enhanced extraction of these compounds at 70°C. The maximum total antioxidant capacity recorded was 12.018 mM α-tocopherol per ml of essential oil. It is noteworthy that different methodologies have been used for DPPH testing and that variations in DPPH concentration and sample volume affect the results as determined by the FRAP and DPPH assays. It is important to note that different methodologies have been used for DPPH testing. Variations in DPPH concentration and sample volume affect the results (Khoshtinat et al., 2016).

Anti-inflammatory activity

ASEO has been shown to have significant anti-inflammatory properties, primarily because it can inhibit the assembly and disassembly of the cytoskeleton (Hussein et al., 2017). Moreover, ASEO has been observed to inhibit the migration of granulocytes and neutrophils into epithelial tissues. Its antioxidant properties further contribute to its anti-inflammatory effects by reducing the activity of NF-κB (nuclear factor-kappa B) in human umbilical vein endothelial cells in the presence of tumour necrosis factor-α (TNF-α). This inhibition occurs through the modulation of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) production, mediated by inactivation of NF-κB (Jeong et al., 2016). The process involves the activation of receptors such as Toll-like receptor 4 (TLR4), which triggers a signalling cascade resulting in the production of inflammatory molecules and cytokines within macrophages. The outcome is a reduction in nuclear NF-κB levels and an increase in cytosolic NF-κB and IκB levels in RAW264.7 macrophages activated by lipopolysaccharides, indicating a decrease in inflammation due to the increased availability of IκB. IκB plays a crucial role in preventing NF-κB from translocating into the nucleus, thereby maintaining NF-κB in the cytosol (Islam et al., 2023).

In addition to its anti-inflammatory effects, allicin, a key compound in garlic, has demonstrated antimicrobial properties by enhancing immune cell function and modulating associated signalling pathways. Allicin also impacts T lymphocytes by inhibiting the chemokine SDF1α, which is linked to cytoskeletal structural weakness and the inhibition of neutrophil transendothelial migration (Sela et al., 2004). The anti-inflammatory activity of ASEO has been shown to affect inflammatory bowel disease (IBD) directly, especially when interacting with cytokines, such as IL-10 and IL-12. It has also been reported to stimulate interferon (IFN) production in T and natural killer (NK) cells (Arreola et al., 2015).

The study reported that ASEO effectively enhanced insulin sensitivity and restored normal serum of nitric oxide (NO) levels, which is indicative of its hypoglycemic potential through the inhibition of pro-inflammatory mediators. However, persistent inflammation can exacerbate insulin resistance, and inflammatory factors such as TNF-α, IL-1, and NO play pivotal roles in this process. The efficacy of ASEO in managing intestinal inflammation is attributed to its bioactive components, including DAS, which inhibits the secretion of TNF and IL-1. Meanwhile, allyl methyl sulfide stimulates the production of anti-inflammatory cytokines. However, persistent inflammation can exacerbate insulin resistance, with inflammatory factors such as TNF-α, IL-1, and NO playing pivotal roles in this process (Zugaro et al., 2023). The efficacy of ASEO in managing intestinal inflammation can be attributed to its bioactive components, including DAS, which has been shown to inhibit the secretion of TNF and IL-1, while allyl methyl sulfide has been observed to stimulate the production of anti-inflammatory cytokines. The combined actions of these compounds can potentially mitigate excess cytokine production during inflammation and suppress pro-inflammatory responses.

Anticancer activity

Garlic is known to contain over one hundred distinct chemical compounds, many of which contribute to the therapeutic properties of ASEO. Key dynamic compounds in ASEO, including DAS, DADS, and DATS, have demonstrated notable anticancer activity (Hussein et al., 2017). At the proper dose, DADS or Allicin can reduce miR-26b-5p (microRNA) levels, resulting in an imbalance in the expression of genes linked with PI3K, AKT, and PTEN, all of which play roles in cell growth and survival. This regulation affects the proteins within the PI3K, AKT, and PTEN pathways, resulting in both upregulation and downregulation. When PI3K and AKT levels decrease, cancer cell proliferation is inhibited, and the increased expression of PTEN induces apoptosis or the elimination of the already growing cancer cells (Islam et al., 2023). ASEO has several ways to fight cancer. It stops cells from growing and spreading. It also controls how the body processes cancer-causing substances. It causes cells to die in a controlled way. And it stops new blood vessels from forming, as well as the spread of cancer cells. All of these things help reduce the harmful effects of cancer treatments (Shang et al., 2019).

DATS, specifically dithioacrolein S-sulfonium, exerts a cytotoxic effect on U937 leukemia cells by generating ROS. These ROS are quite active and can harm cellular components, including DNA, proteins, and lipids. This can lead to oxidative stress and subsequent apoptosis, which is a type of programmed cell death. ROS generated by DATS activate caspases, a group of proteases essential for the apoptotic process, resulting in the disassembly of cellular structures and the death of U937 leukemia cells (Shang et al., 2019). Additionally, allicin, found in fresh garlic extract, has shown effectiveness in inhibiting the proliferation of colon cancer cells. Research suggests that the anticancer action of garlic extract is partially due to its antioxidant and immunomodulatory effects (Hussein et al., 2017).

Glioblastoma multiforme (GBM) is the most prevalent and aggressive type of brain cancer. It often does not respond to treatment and has a poor prognosis. This poor prognosis is partly attributed to the presence of cancer stem cells (CSCs) within GBM and the existence of a chemotherapy-resistant subpopulation of CSCs (Shang et al., 2019). A study has demonstrated that the compound Z-ajoene, which is derived from garlic essential oil, has the potential to hinder the growth of cancerous cells, particularly GBM-CSCs, by exerting an anti-proliferative effect. Remarkably, this inhibition occurred at non-cytotoxic concentrations, indicating that Z-ajoene levels were therapeutically appropriate. Additionally, Z-ajoene has been demonstrated to block the proliferation of human breast cancer cells by inducing apoptosis through increased peroxide production and the activation of caspase-3 and caspase-8 (Zhang et al., 2015).

ASEO toxicity

The United States Food and Drug Administration (FDA) has classified *Allium sativum* L. as generally safe to be consumed by humans. However, adverse effects still exist, particularly when ingested in large quantities or by individuals with heightened sensitivity (Rana et al., 2018). Consuming large amounts, especially when consumed in a fasting state, may disrupt intestinal flora, leading to gastrointestinal issues such as flatulence and other disorders.

Allicin is recognized for its ability to permeate cellular membranes. At a concentration of 200 mg/mL, allicin has been shown to cause significant damage to isolated rat liver cells. Excessive ASEO intake has also been associated with chronic conditions including gastroesophageal reflux disease, kidney hematoma, and an increased risk of pemphigus, an autoimmune disorder (Yogeswari et al., 2020). Oil-soluble allicin is noted to be toxic at higher concentrations than its water-soluble counterpart. However, research by Hizam et al. (2019), found that a dose of 50 μg/mL of ASEO did not induce toxicity or pathological effects when administered to chicken embryos. This was supported by the absence of viral presence in the allantoic fluid of all treated groups.

To assess the toxicity of ASEO, various studies have employed the Brine Shrimp Lethality Test (BSLT). These studies indicate that the toxicity of ASEO depends on factors such as the extraction method, concentration, and garlic source (Waghulde et al., 2019). The LC_{50} is the concentration of essential oil required to kill 50% of brine shrimp larvae within 24 hours. Waghulde et al. (2019), reported LC_{50} values of 10,840 mg/mL for the alcohol extract and 8,180 mg/mL for *A. sativum* L. bulbs. The toxicity of these compounds was tested at concentrations of 1, 10, 100, and 1,000 ppm in a 10 mL seawater solution with 1% (v/v) DMSO. The findings demonstrated that *A. sativum* L. bulbs possess cytotoxic activity against brine shrimp, as evidenced by an LC_{50} value of less than 1,000 ppm or μg/mL, indicating the presence of potent active components.

Strengths and weaknesses of *A. sativum* L. essential oil (ASEO) in bioactivity research

Allium sativum L., widely recognized as a traditional medicinal ingredient, has been used across many cultures and healing systems worldwide (Mugao et al., 2020). Its therapeutic properties are primarily attributed to allicin, a sulfur-containing compound known for its strong antimicrobial effects against a broad spectrum of microorganisms, making garlic a potential natural remedy for various infectious diseases. The antimicrobial efficacy of allicin is linked to its reactive thiosulfinate group, which can interact with thiol-containing enzymes and proteins in microbial cells, leading to the inhibition of vital metabolic functions (Yasin et al., 2022).

ASEO is recognized for its beneficial properties, including antioxidant and anticancer effects (Tanrıkulu et al., 2017). Additionally, ASEO is extensively used in the food industry to enhance flavour and aroma and as a preservative. Despite promising bioactivities, the commercial application of ASEO faces several challenges. One major issue is the variability in chemical composition due to cultivar differences, climatic factors, and extraction procedures. Furthermore, its strong pungent odor and volatility can limit its acceptability in food and pharmaceutical formulations. Therefore, standardization protocols and formulation strategies, such as microencapsulation, nano-emulsion, and solid-lipid nanoparticles, are essential to enhance stability, solubility, and sensory compatibility. The active components of ASEO are highly susceptible to degradation when exposed to light, oxygen, heat, and humidity, which can diminish its dietary and medicinal value (Ezeorba et al., 2022).

ASEO exhibits low solubility in water, which can limit its efficacy in certain applications (Sallam et al., 2004). While ASEO offers numerous benefits, excessively high concentrations can produce a strong and pungent odour and taste, which may negatively impact its sensory qualities in food products. This poses a challenge in optimizing the use of ASEO to maintain its beneficial properties without compromising the taste and overall appeal of food products (Li et al., 2023).

Potential applications of ASEO in the pharmaceutical and nutraceutical industry

The encapsulation technique has been shown to offer a promising solution to the limitations of ASEO, particularly with regard to its solubility and stability. One promising approach involves the use of β-cyclodextrin (β-CD), whose hydrophobic cavity can protect and encapsulate the hydrophobic active components of ASEO, while its hydrophilic outer surface enhances the oil's solubility in water (Khoshtinat et al., 2016). This method significantly improves the utilization and stability of garlic essential oil.

Kumar et al. (2024), reported that encapsulating

ASEO extends its antibacterial effect for up to 265 days at room temperature without loss or decrease in efficacy. This finding underscores the potential of encapsulation to expand the applications of ASEO in various fields, including food preservation and therapeutic interventions targeting a wide range of microorganisms. Furthermore, ASEO contains beneficial compounds, such as DADS and DATS, which have demonstrated potent anticancer properties. However, their limited solubility and stability in aqueous environments pose challenges for their application (Tesfaye, 2021).

To address these challenges, the encapsulation of DADS and DATS within nanocapsules derived from hyaluronic acid derivatives has been investigated. This strategy has been shown to protect DADS and DATS from oxidative damage and adverse interactions with blood and digestive components. It also reduces red blood cell membrane lysis and enhances the stability of these compounds while preserving their biological function and anticancer activity (Souto et al., 2020). The nanoformulation has also demonstrated the ability to inhibit the migration of neoplastic cells, highlighting its potential therapeutic benefits. Improving the stability, delivery, and therapeutic efficacy of DADS and DATS. This encapsulation approach represents a promising advancement in cancer treatment (Yeung et al., 2020).

Research by Hazuka et al. (2021), employed nuclear magnetic resonance (^{1}H-NMR) to monitor the oxidation process of these compounds. The proposed nanocapsule delivery system could achieve higher blood concentrations, prolong circulation time, and enhance therapeutic outcomes. In conclusion, this study emphasizes the transformative potential of nanocapsulation in enhancing the therapeutic applications of DADS and DATS for cancer treatment.

Insight into bioactive compounds

Sulfur-containing compounds such as DATS, AMTS, and DADS are also found abundantly in *A. Sativum* L. essential oil, and it is has reported to exert inhibitory effects on acetylcholinesterase and possess strong antioxidant and antithrombotic properties (Mitra et al., 2022). These compounds are also prevalent in *A. sativum* L., supporting their critical role in the therapeutic mechanisms of ASEO.

The therapeutic efficacy of ASEO is largely attributed to its organosulfur compounds. Allicin, a major bioactive compound, exerts antimicrobial effects by reacting with thiol groups in microbial enzymes, leading to S-thioallylation and subsequent enzyme inactivation. This mechanism disrupts bacterial metabolism and membrane integrity. Similarly, DADS and DATS function through ROS generation and mitochondrial dysfunction, particularly in cancer cells, promoting apoptosis. Understanding these mechanisms is critical for advancing the clinical application of ASEO (Paiva et al., 2014).

Green extraction and future perspectives

In recent years, there has been a shift from classical, solvent-intensive approaches (e.g., long refluxes, hydrodistillation with large solvent/energy footprints) toward greener, more selective techniques for recovering ASEO and sulfur-rich compounds. The concept of "green extraction" is predicated on the principles of reducing solvent toxicity and volume, minimizing energy consumption, and enhancing extract quality by preserving labile molecules. These principles are summarized in foundational reviews of the field (Chemat et al., 2012).

Several particular technologies have demonstrated potential for ASEO and related bioactives. Enzyme-assisted pretreatments, which include cell-wall-degrading enzymes such as cellulases and pectinases, have been shown to enhance the release of oil and volatile sulfur compounds. When employed in conjunction with conventional distillation or steam treatments, these pretreatments often result in a twofold increase in yield. This phenomenon can be attributed to the enhanced disruption of tissue and the subsequent liberation of intracellular alliin/alliinase systems (Sowbhagya et al., 2009). The United Arab Emirates (UAE) utilizes the process of cavitation to achieve several objectives, including the reduction of extraction time, the decrease of temperatures, and the enhancement of the recovery of thermolabile constituents such as allicin and other sulfur volatiles. Numerous optimization studies for garlic have reported higher allicin yields and faster processing times in comparison to maceration or simple solvent extraction methods (Mathialagan et al., 2017).

Natural deep eutectic solvents (NADES) and other green solvents have emerged as flexible media for extracting polar and semi-polar phytochemicals. These solvents are characterized by low toxicity and frequently exhibit biodegradability. NADES can be tuned (choice of hydrogen bond donors/acceptors) to selectively solubilize target compounds, and, when paired with UAE or enzyme treatment, have produced synergistic improvements in yield and bioactivity in recent experimental work. Preliminary studies and reviews suggest that NADES enhance extraction efficiency and stabilize sensitive metabolites during processing (Liu et al., 2018).

Beyond isolated method improvements, hybrid and intensified processes hold particular promise for industrial translation. Examples of such methods include combinations of ultrasound-NADES, enzyme-assisted hydrodistillation, and pressurized or SCF-CO2 extraction for non-polar fractions. The objective of these integrated workflows is to maximize the recovery of specific sulfur species (allicin, ajoene, DATS/DADS) while minimizing thermal degradation and off-odors (Ye et al., 2025). These integrated approaches have demonstrated the potential for enhanced extraction efficiency and reduced environmental impact (Bar et al., 2022). Life-cycle thinking and techno-economic assessments in green extraction literature underscore the necessity of substantiating environmental benefits on a large scale. Key variables in this regard include energy and solvent recycling, solvent toxicity, and downstream purification costs (Ye et al., 2025).

Consequently, future research endeavors aimed at sustainable ASEO production should prioritize the following areas: The first objective is to undertake systematic, head-to-head comparisons of green methods (UAE, enzyme-assisted, NADES, supercritical fluids) using standardized garlic feedstocks and analytical endpoints (profile and stability of sulfur species, bioactivity assays). The second objective is to optimize solvent formulations (NADES components, water content) that both extract and stabilize target volatiles. The third objective is to conduct scale-up studies with solvent recovery and energy balances to validate real-world sustainability claims. The fourth objective is to perform regulatory and toxicology screening of new solvent systems and residuals to ensure food/pharma compatibility. Addressing these areas will facilitate the translation of promising lab-scale gains into reliable, low-impact commercial ASEO products (Bar et al., 2022).

In short, green extraction offers multiple routes to improve yield, selectivity, and environmental footprint in garlic essential oil production-but the most impactful next steps are comparative, quantitative studies that link extraction conditions to the chemical identity and bioactivity of sulfur constituents, and to real-world metrics of sustainability (Chemat et al., 2012).

CONCLUSION

This review provides an integrative and up-to-date synthesis of the therapeutic potential, mechanistic pathways, and technological advancements related to ASEO. The originality of this review lies in its combined focus on bioactivity–mechanism relationships and green technological innovations for ASEO extraction and formulation. *A. sativum* L. Essential Oil (ASEO) has exhibited a range of potentially efficacious therapeutic activities, including anticancer, antibacterial, anti-inflammatory, antifungal, antiviral, and antioxidant properties. A review of the extant research reveals the potential for ASEO to serve as a pharmaceutical agent for a range of medical indications. The mechanisms of action of ASEO include its capacity to scavenge free radicals, thereby reducing oxidative stress, and its effectiveness in disrupting microbial cell membranes, which contributes to its antibacterial and antifungal properties. Furthermore, ASEO has been shown to modulate inflammatory pathways and induce apoptosis in cancer cells, underscoring its potential as an anticancer agent. However, the development of faster and more efficient extraction methods is crucial to ensure the production of specific constituents. Additionally, further exploration is necessary to investigate the nutraceutical bioactivity of ASEO, encompassing its bioavailability and toxicity. In the future, the implementation of green extraction methods and encapsulation technologies offers a sustainable path to enhancing the therapeutic efficacy and industrial applicability of ASEO.

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AUTHOR CONTRIBUTION STATEMENT

DALA and NNI design and concept the study, including methodology and analysis, design and editing MNA, writing and editing manuscript by NNI, DALA, MNA, DR. All authors have reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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