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Biochemical potential of *Salvia sclarea* L.: *In vitro* cultivation and chemical profiling approaches

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Abstract
Salvia sclarea L. is a medicinal plant known since ancient times for its pharmacological potential, due to its rich phytocompound profile, the essential oils, and the secondary metabolites it contains.
In recent decades, there has been an increasingly emphasized trend towards reorientation to natural therapies, leveraging plant-derived pharmacological products, which tend to be milder and have a lower toxicological profile than synthetic counterparts.
Given that the plant's phytochemical profile can vary due to external factors such as soil quality, climate, or season, it is necessary to optimize cultivation methods to ensure the production of plant materials that are rich in active compounds with significant pharmacological potential. This objective can be accomplished through *in vitro* cultures, which enable precise control over growth conditions.
This study is grounded in an exhaustive literature review of *Salvia sclarea* L. and aims to explore its phytochemical composition and pharmacological effects. Additionally, the study investigates its potential for *in vitro* cultivation as a viable solution for standardized and sustainable production of bioactive compounds.

Keywords: *Salvia sclarea* L, *in vitro* propagation, biochemical profiling, phytochemicals

Introduction

Medicinal plants have demonstrated remarkable efficacy over the years, in both the prevention and treatment of various pathologies, playing a crucial role in the development of modern medicine. Numerous pharmacological classes used today have had as their prototype a compound of phytochemical origin, which has served as the basis for developing standard pharmaceutical products. Among these compounds are aspirin, one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs), atropine as an anticholinergic, taxol as an effective chemotherapeutic, and morphine as an analgesic in severe acute or chronic pain [2].

We are witnessing a global resurgence of interest in herbal preparations, driven by their scientific validation and increasing integration into treatment regimens in conventional medicine, along with rising costs in the development of patentable synthetic drugs [2,5].

Salvia sclarea L. is an herbaceous plant, belonging to the *Laminaceae* family and the *Salvia* genus. It is first mentioned as a medicinal plant in the 4th century BC, in the writings of Theophrastus. The name "clary sage" is derived from its historical application in ophthalmology, specifically in clearing foreign particles from the eye. This technique was described by English herbalist Nicholas Culpeper in Complete Herbal (1653) [17,7].

From the botanical perspective, the plant is distinguished by a stem reaching heights of up to 120 cm, branched at the top, and a pubescent covering with gray glandular hairs responsible for the specific aroma. Attached to the stem, the annual leaves are arranged under a rosette-like form, and the biennial leaves are arranged in pairs, their shape is ovate, with a crenate-erose margin, covered with simple and multicellular glandular trichomes. The inflorescence is paniculate, reaching lengths of up to 40 cm, featuring zygomorphic flowers arranged in clusters of 2 to 6. The bracts are characterized by their lilac or white coloration while the corolla display a falciform and bilobed upper lip and a trilobed lower lip with a light lilac or whitish coloration [5].

The fruit is a round or triangular nutlet with brownish seeds with variable shapes [17,7].

The plant is native to southern Europe, it thrives both wild but also cultivated as a medicinal or ornamental plant. This *genus* prefers temperate or subtropical climates with a seasonal variation in temperature and precipitation, with warm summers and mild winters. In Russia, France, Bulgaria, and Morocco the plant is intensively cultivated for its high-demand essential oil [7].

Material and Method

For this study, an extensive analysis of the scientific literature was carried out, focusing on the phytochemical profile, pharmacological effects, and in vitro cultivation methods. Comprehensive searches were conducted in established scientific databases, including articles with rigorous methodologies and original data. Studies without detailed methodologies, original data, scientific relevance, or academic rigor were excluded.

Phytochemical composition

The chemical composition of *Salvia sclarea* L. can vary depending on abiotic factors such as soil type, precipitation, temperature, and altitude.

According to Aćimović et al., 2018, bioactive compounds were found to show concentration variations depending on the geographic region, fertilization, and phenological stage.

The data were collected from 10 studies from 10 different countries, specifically Serbia, Germany, Poland, India, Bulgaria, Turkey, Greece, Iran, Iran, Greece, and Tajikistan, with additional reference data from the European Pharmacopoeia 7.0, (2010). Table 1 shows the minimum, maximum, and average concentrations of compounds obtained from essential oil.

Table 1. Minimum, maximum, and average concentrations of compounds

COMPONENT	MIN (%)	MAX (%)	AVERAGE (%) EO
Linalyl acetate	2.60	56.88	31.07
Linalol	8.50	40.24	23.11
A-terpineol	2.64	14.30	7.03
Geranyl Acetate	1.22	8.37	3.53
Sclareol	0.06	14.62	3.34
Germacrene D	0.52	11.40	2.92
Spathulenol	0.13	19.00	2.16
Caryophyllene oxide	0.20	15.50	2.01
Neryl acetate	0.52	4.69	1.87
Trans-β caryophyllene	0.62	3.41	1.72
B-myrcene	0.20	5.47	1.65
Geraniol	0.31	7.70	1.21
Nerol	0.26	2.54	1.02
A-eudesmol	0.30	2.61	0.78
Limonene	0.20	1.55	0.58
Manool	0.10	3.04	0.57
Cis-β-ocimene	0.10	1.82	0.55
A- and β thujone	0.2	0.90	0.09

The product with the highest therapeutic value obtained from the plant matrix of *Salvia sclarea* L. is the essential oil. It is very rich in linalyl acetate, linalool, (E)-caryophyllene, p-cymene, alpha-terpineol, and geranyl acetate [7].

Mineral content

The leaves stood out as the most mineral-rich part of the *Salvia sclarea* L. particularly high in health-promoting minerals such as calcium, iron, and potassium.

The other parts of the plant are distinguished by the rich content of magnesium and zinc in the inflorescences and sodium in the roots. Table 2 presents the minerals with the highest concentration in *Salvia sclarea* L. and the specific parts of the plant where they are found [3].

Table 2. Minerals with the highest concentration in *Salvia sclarea* L.

Element	**Plant part**	**Concentration (mg/kg)**
Calcium (Ca)	Leaves	17,700
Iron (Fe)	Leaves	2,320
Potassium (K)	Leaves	59,600
Manganese (Mn)	Leaves	78
Cobalt (Co)	Leaves	1
Selenium (Se)	Leaves	0.297
Sodiu (Na)	Roots	873
Zinc (Zn)	Inflorescences	27.3
Magnesium (Mg)	Inflorescences	4,180

The effects of *Salvia sclarea* L. extracts in neurodegenerative and neuropsychiatric disorders

Over the past decade, there has been a significant rise in the incidence of neurodegenerative disorders including Alzheimer's and Parkinson's disease. These diseases are associated with dysfunctions of the enzyme Monoamine oxidase A (MAO-A) which is involved in the metabolization of neurotransmitters in the body, especially the action of serotonin, norepinephrine, and dopamine. In addition to neurodegenerative diseases, MAO-A dysfunction can lead to psychological disorders such as depression and anxiety [22,10].

These inhibitors are among the primary pharmacologic treatments for Alzheimer's disease, depression, social anxiety, and panic disorder. However, in addition to their beneficial effect, they have numerous associated adverse effects including dry mouth, nausea, diarrhea, constipation, drowsiness, insomnia and vertigo [10,20].

It is suggested that antioxidants of plant origin can inhibit MAO action and increase the availability of neurotransmitters improving cognitive function [10]. In the study by Yucel et al. 2023, the antioxidant action and the impact on MAO-A of methanolic extracts from the plant were demonstrated, highlighting *Salvia sclarea* L. as a possible future natural source for the development of therapeutic agents, thus avoiding the adverse effects caused by conventional inhibitors [20].

The effects of essential oil of *Salvia sclarea* L. and sclareol in dysmenorrhea

Dysmenorrhea is a medical condition faced by 1 in 4 adult or adolescent women [26]. It is often ignored or under-diagnosed, negatively affecting women's quality of life, 5% to 14% take time off work or school due to the severity of pain [8,15]. 55.2% of women reported the need for pain management medication for dysmenorrhea to perform their daily activities [8]. The main treatments used for dysmenorrhea are non-steroidal anti-inflammatory drugs and oral contraceptives, in more severe cases, even surgical interruption of pelvic nerve pathways is used [11].

The latest studies demonstrate the efficacy of sclareol in the management of pain associated with dysmenorrhea and uterine contractions. This efficacy is explained by two main molecular mechanisms. On the one hand, by decreasing the activity of OTR (oxytocin receptor) proteins responsible for uterine contraction, COX-2 (cyclooxygenase-2) involved in prostaglandin synthesis and inflammation, MLCK with a role in the involuntary contraction of smooth muscle and p-ERK, p-pp38 and p-MLC20 proteins which have a role in regulating muscle contractions and inflammation through cell signaling pathways. Another molecular mechanism by which sclareol alleviates pain is the modulation of intracellular calcium. Calcium ions play an important role in maintaining smooth muscle contractions. In dysmenorrhea, elevated intracellular calcium levels intensify uterine contractions leading to increased pain. By reducing intracellular calcium, sclareol can help ease uterine contraction and therefore decrease the frequency and intensity of pain [4,28].

Antimicrobial activity

The increasing spread of bacterial resistance to conventional antibiotics is a major problem in the healthcare system, globally affecting more than 100 million people per year [13]. These bacteria are most of the time associated with nosocomial infection. Nosocomial infections are healthcare-associated infections and are one of the most difficult to treat infections, treatment regimens involve complex combinations of antibiotics that can have adverse effects on the body and lead to imbalances in both the gut flora if given orally and the skin microbiome if given topically [16].The most common agents implicated in nosocomial infections are *E. coli, S. aureus*, *Klebsiella* species, *Enterobacter* species, *Enterococcus* species, *Streptococcus* species, and

Coagulase-negative *Staphylococcus* species [19] .The Centers for Disease Control and Prevention (CDC) have estimated that 2 million people worldwide become ill from MRSA infections, 23,000 of whom die from it [23,25,27].

In this context, research in the field is called for to find an effective and lasting solution. One of these solutions may be represented by *Salvia Sclarea* L. oil which, after testing on several antibiotic-resistant strains (penicillin, erythromycin, clindamycin, tetracycline, ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole) has demonstrated its efficacy and antibacterial potential. It had a potent antibacterial effect against all genera of *Staphylococcus* with MIC values between 3.75 and 7.00 µl/ml. Eleven strains of MRSA (Methicillin-Resistant Staphylococcus aureus) and sixteen strains of MSSA (Methicillin-Sensitive *Staphylococcus aureus*) were inhibited by *Salvia sclarea* L. oil [23].

Antioxidant activity

Antioxidant compounds are essential for the normal functioning and adaptation of the plant to the environment, consumed by humans these compounds become beneficial by helping to sustain the integrity of cells against free radicals, cardiovascular disease prevention, prevention of neoplastic diseases, and mediation of immunity [12, 1].

In terms of antioxidant activity Clary sage oil, demonstrated moderate antioxidant activity. It can neutralize free radicals but at a much lower level than Trolox. The DPPH assay had a value of 11.76% and ABTS of 29.70%, where the IC50 value for Trolox was determined as 4.39 ± 0.13 µg/mL for DPPH and 2.96 ± 0.01 µg/mL for ABTS assay [7].

***In vitro* cultivation of *Salvia sclarea* L.**

Medicinal plants traditionally grown in field culture as well as those from the wild flora show limitations and changes in their chemical composition. Secondary metabolites, compounds of therapeutic importance are secreted by the plant to counteract the negative effects of stress.

Limiting environmental factors such as temperature, carbon dioxide, light, ozone, soil water, salinity, and soil fertility have a significant impact on plant physiology [24]. These factors can negatively or positively influence the production and accumulation of secondary metabolites by up to 50%, which emphasizes the importance of implementing a controlled abiotic growth method to obtain bioactive compounds from plants at a detectable concentration [24,18].

In vitro propagation is a method of growing plants in a controlled aseptic environment, free from abiotic stress. This technique can be used to grow crops even in cases where plant species are endangered, produce few or no seeds, or the seeds are sensitive [6]. In addition, plant tissue culture can be used to identify and isolate bioactive phytocompounds from economically important plants with industrial applications in food, cosmetics, and pharmaceuticals [14,9].

Plant propagation is possible mainly by two methods, macro-propagation (in the field) and micro-propagation (plant tissue culture).

Plant tissue culture can be carried out by organogenesis or somatic embryogenesis. There are eight types of tissue culture which are more used:

- Callus culture, a mass of undifferentiated tissue from which specific explants will be obtained, dependent on the action of implanted growth phytohormones;
- Suspension culture, which is represented by friable calli grown in a liquid medium that can be a batch or continuous culture;
- Meristem culture, which utilizes apices with excised lesions having an apical meristematic dome and one or two leaf primordia
- Protoplast culture, which utilizes cells in which the plant cell wall has been removed enzymatically or mechanically;
- Shot-type culture, which in comparison with meristem culture utilizes larger shots and more leaf primordia;
- Lateral bud node culture, involves the use of a short piece of stem with one or more lateral nodes;
- Isolated root culture, which cultivates isolated roots unattached to the buds to obtain a root system;
- Embryo culture using a fertilized or unfertilized zygotic embryo (seed).

In terms of culture media, the most commonly used basic culture media are Murashige and Skoog (MS), modified MS (MMS), B5 by Gamborg, Woody Plant Medium (WPM), and Driver and Kuniyuki Woody Plant Medium (DKW). These media may undergo modification and adaptation depending on the developmental responses of the plant. In addition to the culture medium, a decisive role in plant growth and development, as well as specifically in organogenesis and somatic embryogenesis processes, is played by phytohormones [14,9].

According to Phillips, G. C., et al. (2019), in the case of *Salvia sclarea* L., studies have shown that the suitable medium for *in vitro* seed culture is MS with 3% (w/v), sucrose and 0.8% (w/v) agar. After obtaining explants, these were transferred to MS culture media to which 0.2 mg/L IAA (indole-3-acetic acid), and growth hormones in two different concentrations (1.0 and 2.0 mg/l). The Cytokinins used were, BAP (N6-benzylaminopurines), TDZ (thidiazuron), KIN (kinetin), and mT (meta-topolin). The results obtained support that the effect of cytokinin mT improved the multiplication, growth, and rooting of explants. Furthermore, it was demonstrated that cytokinins promoted the accumulation of diterpenes in leaves and the secondary metabolite profile can be altered by supplementing the culture medium with cytokinins. This suggests that *Salvia sclarea* L. is a promising candidate for future industrial applications of obtaining secondary metabolites [14].

In terms of phytochemical composition, plants obtained in vitro had a lower concentration of oil compared to the amount isolated from *in vivo* plants. However, the concentration of compounds in the oil is similar, thus we deduce that in vitro cultivation does not affect the quality of the oil but only the quantity, without changing the concentration of compounds [21].

Conclusions

Salvia sclarea L. represents a species of interest due to its complex phytochemical composition and high content of minerals and essential compounds which offer notable health benefits. It presents considerable potential in the prevention, treatment, or alleviation of pathologies that are impactful in contemporary settings, including neurodegenerative disorders, menstrual disorders as well as infections caused by multidrug-resistant pathogens.

Moreover, the possibility of phytochemical manipulation of the plant *in vitro*, utilizing phytohormones, allowing to obtain extracts with optimal concentrations of active compounds, makes the plant an ideal candidate for obtaining products of high therapeutic and cosmetic value, able to respond to the current challenges of human health.

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